

## Poor control for US information

*The US Congress has put off legislation on the export of high-technology until after the election. Everybody's interest is that it should be given a clear lead from the administration when it returns.*

THE US Congress, which leaves Washington to fight the presidential election at the weekend, will leave in a mess the rag-bag of unfinished legislation on the export of high-technology with which it has been wrestling for the past two years. And, for the time being, nobody will care. The universities will be for a few weeks free from anxiety about the interpretation of successive draft regulations from the Pentagon. Societies organizing conferences will be able to live for a few months longer with the practices with which they have become familiar on the participation of foreign nationals. Not even the administration will be deeply disturbed, for on the evidence of the opinion polls, there is every chance that it will be invited to return in January with a more compliant Congress, perhaps one less ready to listen to the doubts of those in the universities and industries of the United States, not to mention their opposite numbers elsewhere in the world, who believe that the past year's attempt to prevent the loss of technical information from the United States is inherently misconceived.

Balancing conflicting aspects of the national interest, any national interest, is always like walking a tightrope. Sure feet and physical fitness are essential (which means that governments must be decisive but also capable of administering what they decide). But it is just as important that the balance organs, with or without the assistance of the eyes (Blondel used to walk in a blindfold) should be keenly tuned, sensitive to the dangers of falling on one side or the other as well as to the signs that the body is out of balance (which means that governments regulating the export of technology must know the dangers of miscalculation in either sense). The worry about the past ten years, and the reason why that experience is a gloomy augury for the future, is that the Reagan Administration has shown flaws in all departments.

That a modern state must have a policy on technical secrecy is not, of course, in question. If there were no secrecy, the benefits of military innovation would flow freely to adversaries and, almost as alarming, to states which are to begin with unaligned but to which the advent of new military technology might seem a challenge. When (as in the eighteenth century) the pace of change in military technology was negligible, but wars were so frequent that new technologies were regularly on display, the need for secrecy (or technical espionage) was hardly noticeable. Now, all that has changed. For the past century, military powers have been compelled to increase their efforts to keep their secrets secret, often with remarkable success. The technology of the manufacture of nuclear weapons is even now less widely spread than it might have been, no doubt in part because nuclear weapons states have an interest that there shall not be too many others like them.

### Challenge

The particular challenge of the past few years has been the way in which military and civilian innovations have become intertwined. Even as recently as in the post-war period of 1945-55, when it was already clear that civilian industry had much to learn from the military, civilian innovations were usually by-products of military innovations (jet engines, for example), with the result that the military people were well placed to regulate the speed at which new developments spread. It is too often forgotten that the change which has since come about, only symbolized by the

development of transistors, has radically altered the relationship between military and civilian industry. It is now common for the cost of civil development programmes to be comparable with what the military have to spend. Although, historically, the development of the semiconductor industry based on silicon chips and their successors may have been stimulated by the need to miniaturize rocket-borne equipment, only a few years later the incentive came from the civilian side. In the United States, the process has been accelerated by the practice (constitutionally required) of putting military development out to civil contractors. The result has been a quarter of a century of heady technical development from which both partners have profited in their very different ways.

Two further complications have now arisen. First, as the United States is still somewhat disconcerted to discover, civil industry must make its way in markets which are inescapably international. There is no other means by which the technically innovative companies that keep the United States prosperous can pay for the cost of their innovations. So the knick-knacks which the Pentagon wants to buy turn out also to be on sale outside the United States, and in due course become accessible to other people's Pentagons. This circumstance is, of course, entirely maddening. Why should prosperity, on the face of things a just reward for ingenuity, be inescapably alloyed with the wider diffusion of innovation? And why should salt be rubbed in open wounds when it turns out that some of the beneficiaries of this technical diffusion are not potential enemies but, rather, allies who are even faster at organizing production, or piling one innovation on another? In the strictly commercial field, these are the questions that have so plainly irritated the US Administration in its attempts to win legislation on export controls. The same goes for basic research, where the administration has sought the benefits of openness and the security of selective secrecy. Its judgement has as a consequence been distorted — and its knowledge that something is wrong has undermined its capacity for clear action.

### Muddle

None of this implies that there should be a bonfire of the regulations. Open licences for military equipment would plainly be absurd. So, too, would be open access to military research. The question of where to draw the line between materials that can and cannot be exported must however depend on a judgement of how much time will be spent by military competitors overseas in using similar techniques for the local manufacturer of comparable equipment, and on the likely relationship of that time-lag to the natural pace of development in the field. Nobody who has seen a micro-chip production line at work will think that the sale of a few of the products to a potential enemy is the equivalent of a gift of the technology, but plainly the architecture of a successful computer may be more sensitive. In the old days, merely four years ago, these were the principles underlying the strategic embargo list then in force. By seeking tougher rules without a philosophical basis for designing them, the Reagan Administration has replaced a workable set of rules which were at a few points absurd with a more comprehensive muddle.

What the United States (together with its military allies and commercial competitors, who are mostly the same) is most in need



of is thus an agreed basis on which rules may be worked out as time goes on. The move towards self-regulation by corporations in the United States is a step in the wrong direction — and a recipe for endless uncertainty. The suggestion that there might similarly be a categorization of the research that people attached to universities carry out, with some of it kept secret or semi-secret as other people decide, is not a recipe for “stifling” research but for persuading academic people to pursue uncomplicated goals. On both counts, the result will be less productivity, commercial and intellectual. And the loser will be the United States. Naturally, an openly elected administration is bound to make the choice it thinks the best. On this occasion, unfortunately, the choice is being made irrationally, in the belief that it is possible to keep innovations secret for the rest of time while enjoying the fruits of their sale on the open market. □

## Orphaned academy

*Britain's scheme for a European academy deserves serious consideration.*

THE only squib at last week's predictably somnolent meeting of European science ministers in Paris (see *Nature* 20 September, p. 197) was the British proposal that there should be a European academy of science. And that seems almost to have been an afterthought. The British delegation, embarrassed that its brief called for familiar cautious negativity on any but the most modest proposals for strengthened collaboration, seems hastily to have hit on this wheeze to demonstrate goodwill. It seems not to have known of the European Academy of Arts, Science and the Humanities, based in Paris, to which Nobelists and some others belong. And by not warning them in advance, the British seem to have embarrassed even its friends. Yet the idea is appealing, even to some of those who regard trans-national institutions as an intolerable threat to national sovereignty. For the truth is that the European community of scholarship is potentially much larger than the sum of its parts, many of which are very small, while the cultural diversity of Europe suggests that such an academy would be an interesting institution. What follows is a recipe for those who would put flesh on the bones the British were rattling.

The first step is to make peace with the European institutions that have, in recent years, thought of themselves as embryonic versions of a European academy. CERN, the high-energy physics laboratory at Geneva, was the first in the field, but the visionaries who in the 1950s dreamed of incorporating other fields of scholarship have long since been replaced by people whose chief concern is where the next 100 million Swiss francs will come from. The European Molecular Biology Organisation (not to be confused with the laboratory of almost the same name at the same place, Heidelberg) has also used language of the same kind, and even has a system of elected personal membership built into its constitution. The European Science Foundation (at Strasbourg), potentially the chief beneficiary of the decision of last week's meeting to encourage stronger European research networks, more openly regards itself as the future European academy, but mistakenly. The foundation, which does splendid work on too small a budget, is as it stands essentially a club for European grant-making organizations, at best an academy of academies. As good a case could be made for the Institut des Hautes Etudes, outside Paris, which is supported by the French Government (with modest help from the European Science Foundation) but which is admirably free from chauvinism in its appointment of people. Whatever their virtues, all these institutions lack the essential attribute of an academy — that of being a self-electing meritocracy.

The designers of the new institution will also have to clear their minds about its function. Academies come in several different forms none of which is a ready model for a European institution. The secular function of academies, that of giving advice to governments, conspicuous in the United States and the Soviet Union but less so elsewhere, cannot apply when there is no single government on which to thrust advice. The conduct of research at

a single location is similarly impractical, whatever may have been possible at the academy in St Petersburg in the eighteenth century or in Berlin when the old Kaiser Wilhelm Institute was at its peak. Of the functions of modern academies that could be adapted to a European canvas, the best outlets are the spending of modest sums on the support of scholarship by criteria which are entirely independent, the management of scholarly publications and the pursuit of excellence by repeated demonstration. Such an institution, if properly arranged, could serve to enliven European science in ways not yet foreseen.

But how would it be possible for a European academy to avoid the tokenism to which most kinds of international institutions are prone? Should members of the new institution be elected in proportion to the populations of its sponsor, or to their Gross National Product? Should Nobel prizewinners automatically become members, or be so *ex officio*? Should the presidency rotate among countries? And by alphabetical order or otherwise? Where should the social sciences fit in? Any one of these questions is by itself sufficiently daunting to persuade all but the enthusiasts that the effort would not be worthwhile. There is, however, a way in which these difficulties could be turned. Simply agree that for an initial period of, say, ten years, new members should be elected annually to a European academy by some outside body (for which purpose the European Science Foundation would serve well), but give the existing membership (after the first year) a veto. And then require that for the initial period, no new member should be older than, say, forty. To begin with, it is unlikely that such an organization would be able to do much more than organize occasional scientific meetings. Eventually, however, it might have won enough respect in the outside world, and have learned enough about its own quality, to be able to take independent and less familiar initiatives. And if, by evil chance, luck went the other way, nothing much would have been lost. □

## Redundant department

*Advocates of new US Government agencies include people who should know better.*

RONALD Cape, chairman of Cetus Corporation, president of the Industrial Biotechnology Association and usually a level-headed spokesman for the industry, seems to have been overcome by the excitement of the Biotech '84 conference held earlier this month in Washington, DC. That at least is the kindest interpretation of his talk there, in which he called for the creation of a new federal agency in the United States charged with promoting biotechnology. Its first assignment would be to double the federal government's support for basic research in the life sciences, now some \$2,900 million a year.

Cape is sure to qualify for this year's final round of the Bite the Hands That Feed Them Award with his complaint that the National Institutes of Health (which now spend \$3,800 million a year on research and development, well over half of that on basic research) are too “fragmented” to meet the needs of biotechnology. The other reason advanced by Cape is, of course, the Japanese Peril. The Soviet Peril was enough to bring about the National Aeronautics and Space Administration, after all, and that was nothing compared with the threat of Japanese competition. “They did it to us in cars”, he said. “They did it to us in tape recorders and TVs. And now they say they're going to do it to us again in biotechnology.”

Cape may have been asking for trouble in citing these analogies. Once the Department of Biotechnology is established, presumably with Wally Gilbert as Assistant Secretary for Finance and Mark Ptashne as director of the media relations office, the Department of Automobiles, the National Tape Recorder Administration and the Bureau of Cheap Calculators are sure to follow. To avoid the backlog of proposals that is bound to accumulate, *Nature* would like to put forward its own modest suggestion now: a Department of Magazine Mailing whose major function could be the issuance of grants to worthy journals whose competitive position is being hampered by postal rate increases. □



## Nuclear winter

# US plans for studies proliferate

## Washington

A \$50 MILLION five-year national research plan for studying "nuclear winter" — possible global cooling following nuclear war — has been drawn up by the National Oceanic and Atmospheric Administration (NOAA). A final draft is now being circulated to an inter-agency review committee before being sent to the White House's Office of Science and Technology Policy (OSTP) by the end of October.

The study plan has been drawn up at OSTP's request by a group headed by Alan Hecht, director of NOAA's National Climate Program Office. It seeks to reduce the principal uncertainties associated with nuclear winter by co-ordinated research in several different areas. The \$50 million price tag corresponds to one of several options put forward by NOAA: if the Office of Management and Budget accepts all the study proposals, the cost could be considerably higher. The nominal five years' duration could be extended as necessary.

The proposed study places the highest priority on understanding source inputs of smoke into the atmosphere. Laboratory and field experiments with different fuels would be performed to gain information about the size distribution of smoke particles and their optical properties. Other studies would be designed to investigate how the results from small-scale experiments can be scaled up, first to room size, then to building size and finally to large areas. Ultimately, there would be field

moving equipment and personnel, but some field experiments are thought inevitable. As well as the experimental work, there would be a strengthened modelling effort focusing on further development of mesoscale and cloud models. Optional extras in the plan include studies of the natural transport of Sahara dust and Arctic haze. Also in the high-cost option is work on the biological impact of global temperature changes.

Already, the Department of Energy and the Defense Nuclear Agency (DNA) are managing theoretical studies of nuclear winter. The DNA programme has been allocated \$1.5 million in this financial year and will be increased next year; it will be integrated into the national study if and when that is accepted, when DNA may manage some of the work. But, according to Dr C. Gillespie of DNA, the agency's own work is well under way and will not wait for the national plan. DNA concentrates on the chemistry of smoke and on developing modified three-dimensional global climatic models suitable for nuclear winter studies. It also examines likely explosion scenarios and sponsors policy research. All the scientific work, like that in the national programme, will be published

in the open literature. Gillespie says the only thing clear so far is that the problems are extremely complex.

Academic scientists who have helped plan this study are pleased by the "unusually good co-operation" and the "non-combative attitude" of government officials from all departments. One commented that nuclear winter is a rare example of an area where disagreements between scientists are noisier than those between bureaucrats. But he fears that the administration may seek to use the scientific uncertainties as an excuse to respond only by commissioning research.

Whatever the political complexion of the administration after November's presidential election, a request for some \$50 million of new money over five years will need some justifying. The approval of the President's science adviser (Dr George Keyworth) will be but the first step.

Meanwhile, Congress's Office of Technology Assessment (OTA) is also likely shortly to announce studies connected to nuclear winter. Three broad possibilities are being discussed: a general assessment of what is known about the long-term effects of nuclear war, a survey of all current research relevant to nuclear winter (both in the executive branch and in private institutions) and a more critical study aimed at identifying those areas where further work would be most effective. OTA is unlikely to involve itself in basic research, but feels it does have a role to play in reviewing the efforts of others.

Tim Beardsley

## Netherlands budget

# Under-the-counter support

THE Netherlands Government budget for 1985, announced last week, contains a few grains of hope for Dutch scientists — and there may be more to come. Science minister Willem Deetman, while broadly protecting the budgets of the national research council (ZWO) and academy of sciences (KNAW), has offered them a little more (just one per cent) to pay for new positions (such as predoctoral and postdoctoral fellowships). Meanwhile, he has continued to tighten the noose on the universities, once considered the best-funded in the world. But, it is said in circles close to the government, he is seeking extra funds to pay for the reinstrumentation of laboratories. But why is he doing this outside the budget, who will benefit from it, and by how much? All these questions are matters for speculation.

At ZWO, the small but respected council for basic science, the budget always contains a few per cent extra for re-equipment, but the sum has been too small "for a number of years", says its director, Professor Robert van Lieshout. In the budget this year, ZWO receives Dfl 15 million (£3.5 million) in this category, around seven per cent of the working

budget "but we need ten per cent", says van Lieshout.

Nevertheless van Lieshout is relieved at least to find his net budget uncut. The universities had suffered what they claim to be the maximum possible reductions in recent years, while the research council had been left unscathed; and the Dutch budget minister was demanding more cuts all round. "It's our turn next", said van Lieshout before the budget — but his fears were unfounded.

Moreover, through a budget decision which affects many national institutions, ZWO (and KNAW) find they have a one per cent budget increase to pay for new posts. In ZWO at least, these will be used to provide a few more much-needed postgraduate and postdoctoral fellowships. The Huyghens programme, providing fellowships for high-flyers (20 last year, the first year of the programme), will also be continued by "scraping off" another one per cent of the total ZWO budget. This year, that will pay for around 16 fellowships — although it has been estimated that perhaps another 100 are necessary to maintain the health of science in the Netherlands.

Robert Walgate



experiments entailing managed forest fires instrumented to measure the composition of smoke below, within and above clouds and what happens at the smoke/cloud interface. Some experiments would use areas of clear-felled vegetation.

The number of managed burns the draft plan says, could be contained by studying natural forest fires provided that it is possible to respond rapidly enough in



## Publication controls

# State Department raises hackles

Washington

UNIVERSITIES in the United States are mulling over a new threat to freedom of scientific communication, this time from the Department of State. Recent proposed regulations would restrict the export of technical data "directly relevant" to defence. But there is concern that the regulations could be used indiscriminately to curtail export of any general mathematical or engineering knowledge with possible military applications.

The new proposals are to revise the International Traffic in Arms Regulations (ITAR), which have in the past been used to restrict export of information, although less frequently than the Department of Commerce's export regulations (see *Nature* 20 September, p. 195). The arms control regulations are supposed to apply only to military equipment and data. But while much of the research work covered under ITAR is in any event classified, this need not be so. Thus information "directly relevant" to design, development or operation of defence articles is covered.

However, the progress of the new proposals will be rough. The Department of Defense is piqued that some of its own comments on a 1980 draft of the regulations have been excluded from the text, and is preparing a "statement of non-concurrence". This will ensure that the State Department will at the very least have to work hard to justify its proposals, and another draft may be necessary.

Meanwhile, the Office of Science and Technology Policy (OSTP) at the White House is recruiting prominent scientists and industrialists to form an advisory group on the controls on technical data administered by the Department of Commerce. Its job will be to validate and add political clout to proposals drawn up over the summer by an inter-agency working group under Andrew Pettifor of OSTP, which has rejected an earlier suggestion by the Department of Commerce that the existing general export licence for fundamental research should be removed. Fundamental research is defined as anything done on a university campus, and this group says that fundamental research, together with information in the open literature or mentioned at an open conference, should be freely exportable. Details will be circulated for discussion in a few weeks' time.

So far, the Department of Defense, which was represented in Pettifor's group, appears to be bending over backwards to placate academics. Its new declared policy is that fundamental research should be controlled only through national security classification, in marked contrast to its position earlier this year, when its proposals to introduce a new category of "sensitive" research caused widespread

protests from universities.

The Pentagon will early next month be publishing a list of "critical technical data" that it wants to see protected. But the new national policy, as it has become known,

still has several hurdles to overcome. The proposals of Pettifor's group, which are supposed to provide the nuts and bolts of the new policy, will have to be reviewed both by the White House and by the National Security Council. Any factions in the Pentagon wanting to kill the proposals will still have the opportunity to do so.

Tim Beardsley

## *In vitro* fertilization

# Surrogacy ban by Victoria state

Canberra

THE Victorian state government intends to ban payments for surrogate motherhood and to make surrogacy contracts unenforceable in court following the public release earlier this month of the final report of the committee set up to consider the "social, ethical and legal" issues arising from *in vitro* fertilization (IVF), headed by Victoria Law Reform Commissioner, Professor Louis Waller.

Delivered to the government in August, the report concentrates on the disposition of embryos produced by IVF procedures and should form the basis of widespread discussion throughout Australia. But it approves the techniques which produced the world's first freeze-thaw embryo baby in Melbourne on 28 March this year.

Seven of the nine committee members recommended that the freezing and storage of embryos be permitted to continue, although these procedures should only be undertaken in those hospitals with IVF programmes already approved by the Minister of Health. The hospital itself will not determine the disposition of stored embryos and large embryo banks will not be permitted. Storage, the report says, should be only for short periods, but where a participating IVF couple needs and consents to long-term storage (during chemotherapy, say), storage consent should be reviewed after five years and then may be renewed. The committee also considered that research and development work on procedures for the hitherto unsuccessful freezing and storage of human ova should be encouraged, partly because success would reduce the need for excess embryos in the first place.

According to the Waller recommendations, an embryo should be stored only with the explicit agreement of the couple supplying the gametes, who will have to choose at the outset the embryo's ultimate disposition. If an extra embryo is stored to insure against implantation failure, the couple must decide whether, in the event of a viable pregnancy, their embryo should be donated to another couple in the IVF programme, made available for research or allowed to warm up and perish — to be "removed from storage", in the words of the report. The committee regards the "removal" procedure as morally equivalent to the withdrawal of life-support systems from a mortally ill person.

The embryo should be "removed" if no agreed ultimate provision is made at the time of storage and if, through mischance, the embryo cannot be transferred as initially planned. This appears to seal the fate of the two "orphaned" Rios embryos at present stored at the Queen Victoria Medical Centre in Melbourne, whose parents died last year leaving no directions for the embryos' disposition (see *Nature* 309, 738; 1984). The government will nevertheless allow three months for further public discussion before the Rios embryos are removed from storage. The committee took the view that untransferred embryos should not be regarded as possessing rights or claims to inheritance.

Two committee members expressed the belief that human embryos should never be used in research at all. Another two members — both medical researchers — claimed that the creation of embryos purely for research purposes was ethically acceptable. The other five members, including the chairman, judged that it was not.

They decided that embryological research in Victoria should be limited to the excess embryos produced by patients in an approved IVF programme. Like the Warnock committee set up to examine the ethics of IVF in Britain, the Melbourne group recommended that experimentation on the embryo should extend only over the first fourteen days, and that all embryo research should be regularly scrutinized. A standing review and advisory body or the state health commission are suggested as bodies capable of the task. As a matter of urgency the committee said that the suggested new review body should examine the practice of *in vivo* fertilization which is already employed in a least one infertility clinic in the United States and which one of the IVF groups in Victoria had stated it would be ready to employ if authorized to do so. Other matters it should consider are embryo cleavage, embryo surgery and genetic manipulation.

The committee's terms of reference were limited to surrogacy in an IVF context. The view was taken that surrogate motherhood arrangements are actually agreements for the purchase of a child, and therefore unacceptable. Non-IVF surrogacy is to be covered in the intended contract-voiding legislation which will then not be uniform throughout Australia.

Jeffrey Sellar

## Information technology

## Future reaches Tokyo early

## Tokyo

THE first step towards the creation of the "electronic society" took place in Japan this week with the inauguration of a large-scale model Information Network System (INS) in the Mitaka suburb of Tokyo.

The eventual aims of the INS are grandiose: to provide by around the year 2000 a digital network for the whole of Japan which will integrate telephone, telex, facsimile, teletext, computer data and video transmission systems and enable everything from shopping to business management to be carried out from home. If the network is completed, it will require the staggering investment — at present estimates — of 30 million million yen (US\$150,000 million).

The model system is rather more modest — about 2,000 households are involved at a cost of ¥20,000 million — but it will allow most of the terminal equipment that may be in every household by next century to be tried out. And this stage of the project is going ahead on time, a sure sign that neither criticisms of the INS concept (see *Nature* 305, 364; 1983) nor the impending privatization of NTT, the telecommunications monopoly, has so far made much impact on the project's future.

Among pieces of equipment not before offered to the public are those exploring the possibilities for increased visual communication. There will be video-phones allowing two (or more) people to meet face-to-face on the video screen and a visual circuit service will allow the remote operation of video cameras. This system would, for example, enable an architect to monitor progress on a construction site by dialling the appropriate camera from his home. A totally new visual device is the sketchphone, a telephone that transmits manuscripts and hand-drawn diagrams exactly as they are written or drawn. At first, sketchphones are to be used by the deaf, giving them their first ever opportunity to make telephone calls, and links are to be provided between the Mitaka municipal welfare office and the homes of children with hearing problems.

Other truly new services come from new methods of handling information rather than from new kinds of terminal equipment. If you need to send out the same message to several people, the new network will make that easy: a central voice accumulation device can be used to register the message and the appropriate telephone numbers. The same facility will also be available for facsimile transmission, enabling the chore of sending out New Year greetings cards to be largely automated. The central store may also be used to leave messages for callers, or as a "mailbox" to accumulate calls to be picked up later. Other central facilities will function more like a library. Subscribers

will be able to use a videoreponse system to call up still and moving pictures, or to connect with a pre-programmed motion picture service.

A little more mundane are improvements in existing telecommunications services. Subscribers will have new digital telephones that show the number from which a call is coming, the number being dialled and the call charges. High-speed digital facsimile transmission — including multiple address, mailbox and confidential transmission systems — will be available along with access to the CAPTAIN videotext system. In its digital version, CAPTAIN can offer transmission of still pictures with sound or characters and diagrams with voice instructions for terminal use, as well as devices to turn the picture into hard copy. Thousands of pages of information are already available on everything from chess to weather forecasts.

The real excitement over the next 18 months of the system will come, however, not from contemplating new technology but from seeing whether any really new use can be found for information technology. NTT has selected monitors and information providers in order to put the system to as many practical uses as possible and has also connected the Mitaka network to several businesses in Kasumigaseki, one of Tokyo's main office areas, so that some companies with employees living in Mitaka can try letting them work from home or from a satellite office. Obviously the new high-speed facsimile services and video-phones will come into their own here and may eventually make unnecessary big offices in central areas with sky-high rents.

For the ordinary citizen, it is the combination of facsimile with CAPTAIN videotext that is likely to be attractive. In the financial field, for example, where 74 separate companies are going to provide services, it will be possible for all the operations for which one would normally need to go to a bank to be carried out at home — information on stocks, shares, exchange rates, insurance, and securities can be provided and transactions carried out. Another 248 companies will use the equipment to provide services in a whole range of fields. Department stores will be able to show goods and collect orders, computer shops advise on operation of personal computers, rail and transport companies provide their timetables and route maps, tour agents conduct potential visitors on pictorial tours of countries overseas, oil companies advise of the nearest open petrol station and power companies facsimile their bills straight into customers' homes.

The real hope (for INS believers) is that subscribers will fight their way through the deluge of unwanted promotional material to a whole new range of applications for

telecommunications services. Critics who have looked at the failure of the United Kingdom's Prestel system (which began operation too far ahead of its time in 1979), as well as other nations' viewdata systems, take a somewhat dimmer view. Although INS offers much more than just viewdata, it may still share Prestel's problem of being too stupid to be really useful. Calling up pages of information on a video screen and searching through them is often the slowest and most expensive way to find a particular piece of information, except for special applications such as listing stock exchange prices. What is needed is a truly helpful database, capable of searching for and displaying information specified by its user. No country has yet made available computing power able to handle tasks of that kind.

Whether the Mitaka model system can supply services that customers really want remains to be seen. Those who are desperate for a peek into the future and cannot wait until the end of the preliminary appraisal in 1986 can, of course, simply call up the computerized fortune-telling service that one of the model system companies is offering. And for those who are baffled or simply feel their vocabulary is inadequate for the twentyfirst century, another company is offering explanations of new high-technology terminology as its main service.

Alun Anderson

## Model zoo

## Washington

THE disclosure that a Texan coral snake on display for the past two years at Houston Zoo is in fact a rubber model has caused consternation in the zoo world. A local newspaper was tipped off by an observant zoo visitor who noticed the snake had not moved in 9 months.

John McLain, assistant curator of reptiles at the zoo, explains that coral snakes are difficult to keep in captivity and tend in any case to hide in litter. As they are venomous, the zoo wanted to educate the public in their appearance, so after several real coral snakes died, the model was substituted. Says McLain: "Many zoos make do with photographs, and we thought we were going one better. We didn't think we were fooling anyone." A zoo official denied that any of the zoo's other animals were models.

Dr Dale Marcellini, a herpetologist at Washington DC's National Zoo, says he has never before heard of a zoo passing off a rubber model snake as the real thing before. But he is sympathetic to Houston's problems and thinks it "not such a bad idea". However, Mr Bob Wagner, executive director of the American Association of Zoological Parks and Aquariums, said his association could not approve of the practice. He intends to take up the matter with the director of Houston Zoo.

Tim Beardsley



## Arms control

# More Soviet violations alleged

Washington

AN administration report containing the strongest accusations yet of Soviet violations of arms control agreements may finally be released next week. Hardliners both within the administration and in Congress have been pressing President Reagan for almost a year to turn the document over to Congress. Portions already leaked to the press — presumably by members of Congress who have received briefings from the administration — show it to be a strongly ideological statement that mirrors the view of key administration officials, notably Richard Perle and Fred Ikle of the Department of Defense, that the Soviet Union cannot be trusted to abide by any arms limitations treaties. That view has played a major role in the administration's enthusiasm for its Stars Wars concept of a space-based ICBM (intercontinental ballistic missile) defence.

The report was prepared by the General Advisory Committee (GAC) of the Arms Control and Disarmament Agency, a group of outside experts. Although it is formally a report to the President, both houses of Congress have passed amendments to the defence authorization bill requiring that the report should be transmitted to Congress and the signs now are that the administration will not wait for that bill to be enacted before releasing the report. The visit this week of Soviet foreign minister Andrei Gromyko has apparently delayed an earlier planned release date.

In part, the report echoes the major charges that appeared in an administration report to Congress last January — the construction of a possible ABM (anti-ballistic missile) phased-array radar in Siberia, encryption of ICBM telemetry data, testing of more than one new ICBM and the supposed proxy use of chemical and biological agents in South-East Asia. But the GAC report also chronicles other "breaches" going back to 1958. These include "violations of customary international law" (using booby-trap mines and incendiaries against civilians in Afghanistan) and "breaches of unilateral commitments" (atmospheric testing of nuclear weapons in 1961–62, completing construction of SS-20 launchers between March 1982 and December 1983). One lesser known violation cited is the transit of Soviet aircraft carriers through the Turkish straits, a contravention of the Montreux Convention of 1938.

The GAC report is at its most ideological in six "key findings". The Soviet claim during SALT II negotiations that its "backfire" bomber does not have an inter-continental capability, is, for example, cited as evidence in support of the key finding that the "Soviets use deliberate deception in negotiations". Alleged chemical and biological warfare is evidence

that "Soviets sign and ratify arms control treaties that they are planning to violate".

The report's authors present a novel theory to explain alleged Soviet breaches since signing SALT II. Because these violations were "done in a fashion which should have at least caused US suspicion", GAC concludes that "a reasonable interpretation . . . is that they are measures designed to test US intelligence capabilities and US political processes relative to arms control" and perhaps "preparation or cover for more extensive violations".

Arms control proponents in the United States have recently argued that both the United States and the Soviet Union are "hedging" on compliance. In an article

published in the Fall 1984 issue of *Foreign Policy*, Michael Krepon, director of the verification project of the Carnegie Endowment, notes that the United States is, for example, building a large phased-array radar in Georgia and Texas that could be seen as violating the ABM treaty, as it can cover two-thirds of the continental United States. (The ABM treaty allows only one ABM radar; other radars must point outward to prove that they are early-warning radar only.) Krepon also cites several major weapons construction proposals advanced by the Reagan Administration that would violate existing treaties, including the dense-pack basing mode for the MX, the Midgetman missile and Star Wars. Krepon argues that these actions by the United States signal a "distinct lack of interest in maintaining SALT limitations".

Stephen Budiansky

## Toxic hazards in Britain

NEW safety regulations aimed at protecting British workers from exposure to hazardous chemicals have been proposed by the Health and Safety Commission. The commission, having issued the draft regulations in a consultative document, hopes to elicit comments from interested parties before submitting the proposals to the Secretary of State some time in 1985.

The regulations were prepared by a working party composed of members representing the Confederation of British Industry, the Trades Union Congress and independent experts. They require employers to assess any danger their workers face, to take steps to reduce that danger to an acceptable level and, in addition, to provide safety training, to

monitor the health of workers and to keep detailed medical records. These measures clarify and extend the terms of the presently enforced Health and Safety at Work Act 1974, a piece of legislation dubbed by critics as vague and too concerned with a small subset of toxic chemicals (lead, asbestos, etc.).

Trades unionists are generally delighted with these proposals but some manufacturers believe they will be costly to implement, making British industry less competitive and, consequently, sacrificing jobs. The Health and Safety Commission believes that employers will be helped by a simpler set of regulations applying to all hazardous substances.

Marcus Chown

## Archaeology

# Cheshire's oldest inhabitant?

THE British Museum is about to take delivery of a remarkable archaeological find — the body, preserved in peat, of a 2,500-year-old Iron Age inhabitant. The body, uncovered unexpectedly by peat workers in Cheshire, has been in the mortuary of a London hospital since its excavation last month by archaeologists from the British Museum and the University of Liverpool.

Researchers at Harwell have attached a tentative date of 550 BC to skin samples supplied, though they hope to refine this estimate after careful calibration of the radio-carbon dating procedure. When allowance is made for fluctuations in the atmospheric  $^{14}\text{C}/^{12}\text{C}$  ratio over past millennia, the technique could possibly yield an earlier Bronze Age date for the body.

Dr Ian Stead of the British Museum's Department of Prehistoric and Romano-British Antiquities is to supervise the work on the body. Until the layers of peat are peeled away, the exact state of preservation

of the body will not be known. But there are high hopes that, apart from the skin already found, body hair, clothing and jewellery will also be revealed. With luck it may also be possible to inspect the stomach contents.

This is the first such find in Britain, though similarly preserved bodies have been discovered in Jutland, Denmark. The Danish remains showed signs of violent sacrificial deaths and have ranged in age from 840 BC, the end of the Bronze Age, to as late as AD 95.

Dr Stead believes that, because preservation techniques have become increasingly sophisticated in recent years, the British find could prove the most important yet. The last Jutland body came to light in 1952 and its condition has deteriorated over the years. The British Museum intends to use the more effective technique of freeze-drying. The museum hopes to have its body on display to the public, curled in its original fetal position, within two years.

Marcus Chown



## UN biotechnology

## Green light for twin lab

THE plan to found a biotechnology organization on behalf of developing countries, masterminded during the past two years by the United Nations Industrial Development Organization (UNIDO), moved one notch ahead last week. The preparatory committee of the International Centre for Genetic Engineering and Biotechnology, meeting in Vienna, heard for the first time what funds the two host countries, India and Italy, are willing to commit. The plan now is that a project leader and a scientific committee should be identified by the committee's next meeting in December.

Opinions are divided about the pace of events beyond that. The two years already spent on the discussion of the project are not thought excessive by the yardsticks of international diplomacy, and because the end-product will be a free-standing international organization to which the 33 member states will be bound by a convention with the legal force of a treaty.

In many cases, the project became tangible only at the beginning of this year, with the decision that the new centre would have two components, one in Italy (Trieste) and one in India (Delhi).

The funds now allocated by the two host governments are substantial but far from sufficient to keep the project going. The Government of India last week offered \$5 million towards the cost of the first five years' work at the Delhi site, supplemented by an offer of \$8.2 million from the Italian Government, which has also promised \$4 million and 13,000 million lire towards the cost of five years' work at Trieste.

The two governments will also contribute towards the cost of equipment (Rs 15 million and 58 million lire for India and Italy respectively). India plans to spend Rs 72 million on land and buildings near Delhi, while Italy will provide a building at Trieste at a token rental.

These bids will demonstrate the seriousness of the two hosts and also serve to encourage the others, as at a charity auction. Whether the other potential contributors will arrive at Vienna with enough promissory notes is, however, less certain. Some will wish to know what people are chosen in the next three months, others the colour of their fellow-members' money, while doubts persist about the decision to split the centre into two components.

A further difficulty is that the centre is planned to be the hub of an international network of "associated centres" that will be nationally located so as to assist with training and exploitation. The obvious danger is that member governments will prefer to commit what funds they can afford to the development at home.

There are also further decisions to be made about the division of work between Delhi and Trieste. The Indian centre will

be concerned with developments in agriculture and human health, the Italian with the application of biotechnology in the chemical industry, energy and the like. But where (and how) the dividing line will be drawn is still to be decided in detail. □

## Badgers at bay

THE conflict between British farmers and wildlife interests over the badger as a source of bovine tuberculosis will persist, last week's report on the subject from the Ministry of Agriculture, Fisheries and Food (MAFF) notwithstanding. The issue is contentious because the badger is one of the largest native mammals surviving in southern Britain and because wildlife interests dispute the reality of the connection between badgers and bovine tuberculosis. Under present policy, badgers are protected under the Badger Act except in areas (mostly in the south-west) where infection by *Mycobacterium bovis* and badgers coexist, as recommended by Lord Zuckerman in 1980.

The latest report on *Bovine Tuberculosis in Badgers*, the eighth to be published, extends the study of captured badgers up to the end of 1983. It says that 13 per cent of 6,000 carcasses examined at MAFF laboratories between 1971 and 1983 show traces of infection, a higher rate of incidence than in any other common British animal.

But do badgers communicate the disease to cattle? If so, then it is presumably via faeces. Tests for *M. bovis* in faeces over the same eleven-year period were positive in 3 per cent of cases. The evidence implicating the badger population as a reservoir for *M. bovis* remains circumstantial and rests on the coincidence of outbreaks of bovine tuberculosis with concentrations of badgers. The case against badgers is strong but not watertight.

There are, unfortunately, no reliable estimates of the size of Britain's badger population. It is unlikely, the World Wildlife Fund believes, that the species is threatened nationally by culling.

Live trapping has now replaced gassing as the chief method of controlling tuberculosis in badgers, in spite of the extra cost. All cattle are tested regularly for bovine tuberculosis and those reacting to the tests are slaughtered. Research into the link between bovine tuberculosis and badgers and control of the disease continues.

Meanwhile, MAFF has announced a further comprehensive review, as recommended by the Zuckerman report, of the problem of badgers and bovine tuberculosis, to be carried out by a three-man team under Professor G.M. Dunnet (University of Aberdeen), and the ministry now promises that the new findings will be made public.

Marcus Chown

## Sellafield

## BNFL on counter-attack

BRITISH Nuclear Fuels Ltd (BNFL), the focus of public criticism during the past year over its waste-disposal indiscretions, has hit back at its critics in its annual report, *Radioactive Discharges and the Monitoring of the Environment, 1983*. The company hopes that data published in the report will draw the debate on its conduct out of the emotional arena and back into the forum of facts. But this seems unlikely while the argument about high leukaemia incidence near BNFL's Sellafield reprocessing plant continues.

BNFL reports that, during 1983, the quantity of liquid waste discharged from its Sellafield site into the Irish Sea was reduced substantially, continuing the trend of recent years. The total beta discharge was cut by 50 per cent compared with the 1982 level and the alpha discharge was down by 30 per cent. These reductions were achieved despite the contamination of a 15-mile stretch of the Cumbrian coastline, near Sellafield, in 1983 after the accidental transfer of plant washings to a sea tank on site, for which the company is now to be prosecuted. BNFL says that the total radioactivity released as a consequence of this error was negligible, amounting, over an eight-month period, to less than one-tenth of a curie (1 curie =  $3.7 \times 10^{10}$  disintegrations per second). This is to be compared with Sellafield's routine beta discharge in 1983 of 67,000 curie.

BNFL concedes that the contaminated beaches seriously damaged public confidence in the company. To restore that confidence, it is to step up its research efforts to cut liquid discharges to "essentially zero". £190 million is already earmarked for this purpose which will, of course, require an alternative disposal route, most probably to a land site. The company stresses, though, that it now operates well within the government's limits for the dumping of liquid waste.

BNFL has continued its programme of monitoring the environment during 1983. Shellfish eaters near the Sellafield plant have been identified as potentially at risk from radioactive contamination, but tests have verified that their exposure amounts to about one half of the annual limit recommended for members of the public by the International Commission on Radiological Protection (ICRP). According to the report, no other group in the vicinity of any other BNFL installation receives an annual dose even close to this level. These findings are confirmed by an independent study carried out by the Directorate of Fisheries Research of the Ministry of Agriculture, Fisheries and Food (*Radioactivity in Surface and Coastal Waters of the British Isles, 1982*).

Marcus Chown

## French research

# Pragmatism back in fashion

### Paris

DURING the past year of economic crisis, the smile on the official portrait of President Francois Mitterrand has cast a rather baleful air over government offices. But now that smile is visibly brightening. Mitterrand's new boy, 38-year-old Prime Minister Laurent Fabius, is turning France from socialism to social democracy. Mitterrand looks happier with the new tag, and science may benefit as well.

Fabius's minister of research, M. Hubert Curien, is a technocrat, not a politician, and will not let ideology get in his way. Asked by a newspaper last week to choose his *leitmotif*, he replied "to choose and to act". This may sound banal, but Curien means it. And there is no time to hesitate. General elections are due in 1986, and the electorate has lost its taste for the *Partie Socialiste*. The President needs results.

While the French plan — to base economic growth on research — has life in it yet, the rose-tinted spectacles are gone. There is a new sense of realism, for example, on the French policies on the new technologies, in the past always suspect because they were so centralist (despite efforts to the contrary).

French centralism works beautifully in projects which require the mobilization of France like an army, as Napoleon knew. Thus the French have built the TGV (high speed train), conquered space (with Ariane) and introduced nuclear power (half French electricity is nuclear, and the proportion is rising). The recipe has not worked as well for the multifarious worlds of biotechnology and the microchip. Central projects in biotechnology and electronics launched two years ago have run into the sand.

The big surprise is that technocratic centralist France now admits this. The Prime Minister is now being told influentially that these projects must now be decentralized, made more responsive and various. One lesson learned is that while it may be logical for the Institut Pasteur to have just one company — Institut Pasteur Production — effectively controlling applications of Pasteur research, and for the medical research council INSERM to have a similar body controlling applications of monoclonals (Immunotech), commercial and practical realities make such unique relationships inefficient. So change may be expected there, and in many other places where centralism and "logic" have prevailed.

It helps that Curien has room in which to move. His ministry (research and technology) is rid of the short-term problems of heavy industry, which had begun to dominate the old ministry of industry and research in Fabius's time. But useful links between the industry and research administrations have been retained. Curien's

budget has also sensibly increased, and there is a prospect of his having more direct power over the budget of a number of organizations (such as the council of agricultural research, INRA), previously only influenced by the ministry.

One immediate task for Curien's ministry is to prepare the budget for 1986, the first year beyond the 1982–85 "law for research" defined by the first socialist science minister, M. Jean-Pierre Chevènement. That could be important. The law as it stands ties political hands. Curien may wish to try something new, such as abandoning the *programmes*

*mobilisateurs* which centralized policy in, for example, biotechnology and electronics.

Another hopeful sign is that French science has loosened up over the past two years. People are more aware of the outside world and more willing to change where change is needed. Even links between research and industry have improved — from a very low base. According to Philippe Lazar, director-general of the medical research council INSERM, "there's been a real shift in the ideology" both in industry and research.

So Mitterrand's new government has brought a sense of hope. But the Fabius honeymoon will soon be over. Then the marriage must begin.

Robert Walgate

## UK biotechnology

# Advertisement sails near wind

AN advertisement by the biotechnology group Porton International that appeared in *Nature* on 30 August (and in the US journals *Science* and *Bio/Technology*) appears to have caused a flurry of confusion, even discontent, about the relationship between the company and the Centre for Applied Microbiological Research (CAMR) at Porton, the British government laboratory converted from defence to civil purposes five years ago.

Other commercial companies are offended that the advertisement may have been taken to imply that CAMR is either a subsidiary of Porton International or that it may have an exclusive commercial relationship with Porton International. The Public Health Laboratory Service, whose chief function is to provide pathology services and research for British health services, says it wishes to "make it quite clear" that CAMR is "in no sense a subsidiary company of the advertiser".

Even before the transfer of CAMR from the British Ministry of Defence to the Public Health Service Laboratories, CAMR had been keenly interested in the commercial exploitation of some of its projects, notably that for the production of asparaginase used in cancer chemotherapy. Part of the case for not closing the laboratory five years ago was the argument that the British public investment in biotechnology required a laboratory with Porton's experience in microbiology.

The advertisement has given offence because CAMR is described as a component of several of the company's "ten divisions", in which connection it is listed together with operating subsidiaries of Porton International, and because of the use of an aerial photograph of the CAMR laboratory block and another of a new building as "the new production centre at CAMR, Porton". Complainants say that the achievement gives casual readers the impression that the facilities at Porton are the property of Porton International and

not the British Government. The advertisement does, however, say (on the same page) that Porton International "has the largest commitment of any company to research and development at CAMR", implying that the relationship between the two organizations is not exclusive.

Porton International, described in the advertisement as an "independent private group whose shareholders include world leading pension funds and institutional investors", is a company recently created by Wesley Haydon-Baillie with funds from UK and overseas financial institutions. Its British subsidiaries include the well-established company LH Fermentation. Porton International also has a licensing agreement on the herpes vaccine developed jointly at the University of Birmingham and CAMR, now in clinical trials, while other collaborative ventures are in the process of being negotiated.

The laboratory staff's views of the advertisement are mixed. Some point out that Porton International is the largest single customer for CAMR's research services, in which role it supports several members of the laboratory's staff and also has a lien on a proportion of the potential output of the production plant. Others, however, still resent the company's use of the name "Porton", first registered for commercial use in 1982.

By some accounts, the appearance of the advertisement may have complicated a series of negotiations which have been under way for some time between the Department of Health and a number of British companies with interests in biotechnology, the chief effect of which would be to convert part of CAMR into a research facility for a commercial consortium. This is not inconsistent with the insistence of the Public Health Laboratory Service that CAMR is independent and "that there is every intention that this independence should continue".

John Maddox



## Florida citrus

# Burning bugs sole defence

### Washington

WITH the discovery last week that citrus canker has spread to four new nurseries in Florida, drastic measures to halt its further spread seem unavoidable. State agricultural officials have been granted authority to burn all infected trees as well as all trees standing within 125 feet of any tree originating from one of the five infected nurseries. The nursery where the outbreak was first discovered, and which appears to be the source of the other outbreaks, has shipped 89,000 trees since January this year.

Meanwhile, a handful of scientists in Florida and at the Department of Agriculture (USDA) Beltsville laboratory, as well as its Animal and Plant Health Inspection Service, are struggling to discover where the infection originated and whether controls more sophisticated than burning can be effective. According to Dr Ed Civerolo of Beltsville, who has studied citrus canker since 1978, the bacterium responsible for the Florida outbreak appears to differ from those found in recent years in Mexico, Brazil, Argentina and Japan. Although of the same species (*Xanthomonas campestris citri*), the bacteria in different areas exhibit different host preferences and virulence. The Florida bug is the most virulent, attacking all citrus species. Significantly, the Florida strain does not resemble the Mexican, which is a relatively mild version and which is specific for key lines. Initial studies by Civerolo over the past few weeks indicate that unlike all other known strains, the Florida version contains no plasmids; it also differs in fatty-acid composition and is serologically distinct.

Citrus canker last appeared in the United States in 1912, when it took 15 years to eradicate. On that occasion, the bacteria were carried on rootstocks imported from Japan.

Surprisingly, burning is still the only effective control measure. Bactericides have been singularly ineffective in fighting bacterial plant diseases of all sorts; under favourable environmental conditions (such as a hot moist climate and extensive acreages of suitable hosts), the bacteria simply grow faster than they can be wiped out. Bacterial resistance to antibiotics is also a problem.

An epidemiological study is now beginning to trace the source of the latest outbreak. Contaminated growing stock is the most likely source. Although the United States forbids importation of growing stock except for research (and then only under strict quarantine which requires isolation and propagation in greenhouses for a matter of years), USDA officials say they believe a certain amount of stock circumvents the safeguards, entering the country illegally. Since the

bacteria infect stems, leaves and fruits, it is also possible that they were carried in on contaminated fruit or other debris that somehow came in contact with the Florida trees.

The United States has maintained quarantines against the importation of citrus fruits from other countries where the disease is present. So far, none has reciprocated following the Florida outbreak. The US Government has, however, imposed an interstate quarantine; only fruits from inspected groves will be permitted out of Florida, and then only if they are dipped in a chlorine disinfectant and if the destination is a non-citrus-producing area. (Texas and California are

the other major citrus-growing states.) The most immediate economic effect on the Florida growers, however, is likely to be that of the eradication programme. Compensation is not being provided to those whose trees are destroyed, and the US Secretary of Agriculture could order compensation only after declaring an "extraordinary emergency", which has never been done for a plant disease.

If the infection should become established in Florida, that would not mean the end of the Florida citrus groves. The immediate consequence of infection is disfiguration of the fruits by raised brown spots and a decline in vigour of the trees and of their yield. But in severe cases, substantial defoliation and premature fruit drop can occur, and the trees may ultimately die.

Stephen Budiansky

## Danube environment

# Hungarians seek outside support

### Budapest

THE Hungarian Government has an environmental battle on its hands over a controversial hydroelectric scheme which, among other things, would entail the diversion of the Danube. Ironically, while the fourth environmental engineering exhibition was being mounted last week, an unofficial Hungarian group called the "Danube Circle" began soliciting Austrian support for its opposition to the project.

The project, which includes the diversion of the major part of the Danube waters through a canal to a power station at Gabčíkovo in Czechoslovakia, and the construction of a dam at Nagymaros which would flood the most scenic stretch of the Danube, has been undertaken jointly by Czechoslovakia and Hungary. Hungarian environmentalists fear that the dam would cause irreversible damage to the water table of northern Hungary, endangering the water supply of up to 8 million people, and could lead to the disappearance of many species of flora and fauna from the river and its banks. The agreement between the two governments was signed in 1977, but was based on plans drawn up much earlier, when the long-term environmental consequences were little understood.

Between 1978 and 1983 there was considerable criticism of the scheme in the Hungarian media. Political considerations made it impossible for the Kadar government to withdraw unilaterally from the scheme; nevertheless, in 1981, all work on the Hungarian side stopped, allegedly due to lack of funds. Last autumn, moreover, while signing a new agreement reiterating Hungarian commitment to the scheme, the Kadar government managed to reschedule the completion date from 1990 to 1994.

At this point, the Danube Circle launched a petition, signed not only by Hungary's small community of "dissidents" but also by a number of

leading environmentalists, some of whom had actually worked on the official survey of the hazards.

The protesters were particularly irate that the Hungarian public had never been allowed to express an opinion about the project, and that, furthermore, no proper survey had been made to establish the global effects and risk factors. The government responded by clamping down on all media discussion of the project — a somewhat unusual response for Hungary, which in general prefers to stimulate public discussion of environmental issues, so as to bring fears into the open and then allay them with a concerted press campaign.

Upstream, meanwhile, an Austrian proposal to build a similar dam at Hainburg provoked a protest petition with more than 150,000 signatures. Presumably in response to this, the Austrian Government proposed an arrangement by which it would partially finance the construction of the Nagymaros power station in return for a supply of power in the future. But according to the Danube Circle, the whole concept of the scheme, which is to top up electricity supplies at peak hours, is impractical, since the Danube is at its lowest in winter.

It is rumoured in Hungary that the government has had second thoughts on the affair and is trying to find a diplomatically acceptable way of withdrawing. (This view is perhaps substantiated by the fact that the International Danube Commission, based in Budapest, is likewise unwilling to comment on the situation, implying that some kind of negotiations are taking place.) If, however, Mr Kadar has been using the excuse of lack of funds to postpone construction works until such a solution can be established, the Austrian proposal to contribute to the construction costs could prove embarrassing.

Vera Rich

# Objectivity versus doctrine

**SIR** — It has been fashionable for scholars on the periphery of science to attack the idea that science can be objective and value-free. This view stems from confusion between different meanings of the word "science" as a highly subjective activity, a methodology that maximizes objectivity and the resulting body of knowledge. Although we have become more aware that unconscious bias is often hard to avoid, the goal of objectivity remains the foundation on which the success of science rests. No less than in Galileo's day, the deliberate introduction of ideological preconceptions into the scientific process undermines its integrity.

I was therefore dismayed that Sir Peter Medawar reviewed *Not in Our Genes*, by Lewontin, Rose, and Kamin, with enthusiasm (*Nature* 19 July, p. 255). Moreover, he even quoted approvingly part of the authors' frank statement of political purpose: "We share a commitment to the prospect of the creation of a more socially just — a socialist — society. And we recognize that a critical science is an integral part of the struggle to create that society, just as we also believe that the social function of much of today's science is to hinder the creation of that society by acting to preserve the interests of the dominant class, gender, and race." Unfortunately, in admiring the goal of a just society, Medawar overlooks the possibility that the authors' commitment to Marxist doctrine would conflict with their commitment to objectivity.

In fact, the authors skilfully distort the views of scientists interested in human behavioural genetics, intelligence testing and sociobiology, condemning research in these areas as worthless rather than as necessarily limited in precision. Indeed, almost no biomedical research could meet their perfectionist standards. Moreover, they project upon their victims a political motivation equal in intensity, but opposite in direction, to their own. They even condemn Kety's classic demonstration of a major role of heredity in schizophrenia, ignoring the fact that genetic studies on this disease are likely to lead, through the reductionist molecular genetics that they decry, to specific chemical therapy.

Historically, this book must be regarded as part of the long campaign of a group from the radical left, called Science for the People, to outlaw the study of human behavioural genetics. While most of the book repeats this group's earlier arguments, there is one major shift. After many years of trying, with little success, to convince the world that genes have little to do with individual differences in behaviour or in potential, Lewontin *et al.* now deny any such naive cultural determinism and adopt the position long held by their opponents: intelligence is the product of gene-environment interactions. But their

turnabout seems to be more a matter of strategy than of conviction: note the title of the book, and the statement that J.B.S. Haldane and H.J. Muller "argued (along lines that we would not) that important aspects of human behavior were influenced by genes" (p. 73). Moreover, instead of gracefully seeking an end to the sterile polemics, they claim the high middle ground of interactionism for themselves, and they cast down their opponents with the epithet "biological determinist" repeated (in a familiar political tactic) on virtually every page.

The authors are unusual not only in the righteousness but also in the manners that they bring to scientific controversy. For example, after quoting Louis Agassiz's assertion (in the nineteenth century) that the human sciences can in principle be freed of politics and religion, they add that "The sentiment was echoed in 1975 by yet another Harvard professor and biological determinist, Bernard Davis, who assures us that 'neither religious nor political fervor can command the laws of nature'". Then follows another quotation from Agassiz: "the brain of the negro is that of the imperfect brain of a seven-month infant in the womb of the white".

As a reviewer has noted, this slur, passing three authors and an editor, says much about the intent of the book (see M. Konner, *Natural History*, August 1984, p. 66).

Why does such doctrinaire and ambiguous rhetoric appeal to many thoughtful people, as it has to Medawar? Obvious reasons include the past misuse of genetic theories to support racism, fear that genetic studies of behavioural traits might reveal differences between races and the belief that it is racist even to entertain that possibility. But this belief, however well intentioned, is profoundly illogical: racism is the willingness to have race, rather than individual qualities, determine a person's social treatment. Moreover, modern genetics has made a major contribution to the struggle to overcome this evil. For the intellectual justification of racism has been the ancient assumption that the differences between races are typological — that all the members of one group differ from all the members of another. But in contrast to the naive support of this view by certain geneticists in the past, modern population genetics has utterly destroyed its typological foundation. We now know that the genetic differences between races (except for some physical traits subject to climatic selection) are statistical and overlapping, and so one cannot infer an individual's potential from his race.

Future advances in understanding human diversity could also help us to reach humanitarian goals, by improving our efforts to develop individual potentials. To be sure, since knowledge of genetic

differences, like almost any scientific knowledge, could be used for ill as well as for good, it is understandable that some people focus on the immediate danger. But the shift of genetics from typological to populational thinking encourages confidence that its further advances can have a constructive social impact.

It is sad to see one with Medawar's eminence, and with his credentials as a writer on the philosophy (and on the manners) of science, lending credibility to this book. He does criticize its attack on reductionists, because he finds that they simply do not exist as described. But he does not question the existence of biological determinists, even though they are equally imaginary among modern biologists. He also approves what he terms the "right-thinking" quality of the book. But "right-thinking" (or orthodoxy) is a dangerous concept in matters involving science. If it refers only to the general goal of a just society, or to Medawar's stated belief that the world is in need of change, fine — but if it encompasses or condones political bias in the evaluation of science, it presents a grave challenge.

The extreme positions and the political propaganda presented in *Not in Our Genes* are unlikely to influence the views of many scientists close to the field. Nevertheless, the book may convince lay readers, and it will surely have a distorting influence on the public image of science.

BERNARD D. DAVIS

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## Genes-on

**SIR** — We now have quite a large number of types of gene. It might be sensible to suggest they are all named in the same way, so that they can be instantly recognized, as enzymes are with their "ase". Maximum simplicity and clarity may be achieved if they all ended with "on", with enough on the front end to indicate their function or status. We have already: recon, muton, replicon, codon, cistron, operon, transposon, regulon, exon, intron operon. There must be others I have not thought of.

Recently a new gene with a widely conserved sequence which may control development in a wide range of segmented animals has been announced, how about "onton"? This would go well with a single oncogene being called an oncon.

Other conversions could be: plasmogene into cyton for cytoplasmic genes; palaeogene into palon; neogene into neogon; regulator into represson (distinct from regulon which means something else).

Perhaps a modern geneticist should define all these terms as proper units, so that we may all express ourselves with precision.

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# Mistreatment of laboratory animals endangers biomedical research

from Christine Stevens

*There is now an opportunity for legislators in the United States to rescue medical researchers from themselves.*

In the United States, laboratory animals are used in much greater numbers and subjected to far more neglect and mistreatment than elsewhere, in Britain for example. The reason for this is simple: US law does not come to grips with pain prevention. In the absence of an adequate regulatory regime, an all too casual attitude has developed. Pain relief, or indeed any sort of post-operative care, is often omitted in laboratories, test procedures are unnecessarily duplicated and even the minimal standards of care required by current law are routinely ignored. These negligent attitudes are reinforced by a chronically short-staffed and underfunded federal enforcement programme on the one hand and, on the other, an aggressive lobby of commercial animal suppliers whose objective is, simply, to sell as many animals as it can to researchers and testers.

## New Bills

An opportunity has now presented itself to bring the federal Animal Welfare Act up to a level roughly comparable with the laws governing the use of laboratory animals in Britain and fourteen European countries. Congress has been presented with two legislative proposals, the Senate with a bill (S.657) introduced by Senator Robert Dole (Republican, Kansas) and the House of Representatives with one (H.R. 5725) introduced by Representative George Brown (Democrat, California), both of which have been endorsed by the American Veterinary Medical Association. Unfortunately, opposition organized largely by the trade association representing laboratory animal breeders has so far prevented these proposals from getting anywhere.

There is no shortage of examples of the current act's deficiencies. The only pain-reducing provision is a deliberately vague phrase included under a general requirement for "adequate veterinary care" which calls for "appropriate use of anaesthetic, analgesic and tranquillizing drugs". Public scrutiny of compliance with this provision is provided only by a requirement that laboratories list the numbers of painful experiments and tests conducted without such drugs, and then note the reason in a one-page annual report.

As a result, compliance even with this vague requirement has been casual in the extreme. The majority of medical schools boldly claim, year after year, that all the pain and distress they cause experimental animals is prevented by pain-killing drugs.

(Pharmaceutical houses tend to be more honest in admitting that they inflict suffering; this they usually blame on the testing requirements imposed by the Food and Drug Administration.)

Under the current law, it is entirely up to the researcher to determine whether pain-relieving drugs are necessary. Edward Taub, who received much publicity after the National Institutes of Health (NIH) suspended support for his research on deafferented monkeys, claimed that his superior knowledge made veterinary advice unnecessary. He claimed that no pain-relieving drugs were needed because sensory nerves were cut in the procedure he was using. But Dr William Pryor, the laboratory veterinarian at East Carolina University, testified before a Health and Human Services Department appeals panel to which Taub had taken his case that analogous procedures on humans result in great post-surgical pain, and he recommended an analgesic specifically suited for the purpose.

At the University of California at Berkeley, researchers charged that graduate students conducting major surgery, including the removal of eyes from cats, in Dr Russel DeValois's laboratory, often administered anaesthesia incorrectly. One witness said, "From the high-pitched squealing of the kitten, it was obvious that the animal was experiencing intense pain".

The Dole/Brown proposals would impose the very modest requirement that researchers consult with a veterinarian in the planning of any procedure involving pain to unanaesthetized animals. The bills would also require that post-operative care be provided and that proper staff be available at nights and on weekends. This might do away with the scheduling of surgery at entirely inappropriate times, as happens now. I have seen unconscious post-surgical dogs left in a room with twenty other barking dogs at 3.30 on a Friday afternoon "Been doing some cutting today?" the veterinarian said to a caretaker down the hall. "Yeah", he nodded.

## British act

Before giving further examples to illustrate the need for improved legislation in the United States, I should note that about thirty years ago, I went to Britain at the suggestion of my father, Robert Gesell, Chairman of the Physiology Department at the University of Michigan Medical School, to consult those whom he considered the best biomedical scientists to get their views on

the British act regulating animal experimentation. I spoke to Sir Alexander Fleming, J.B.S. Haldane and many other distinguished investigators, and visited their animal rooms.

Even though funds were extremely limited at the time (just after the Second World War), I found the animals far better housed and looked after than in most of the American universities I have recently inspected. Fleming's rabbits were fine-looking creatures, at ease in cages big enough for them to take a hop or two and sit up in the classic Easter rabbit pose if they felt so inclined. Haldane's newts lived in large well-furnished aquaria. In the dog laboratories I visited, the dogs occupied good-sized pens — in sharp contrast with the small cages stacked to the ceiling in many US dog rooms of the period, where the cages were commonly hosed with the dogs still inside trying to dodge the stream of water.

No scientist I interviewed during my visit complained of the British Cruelty to Animals Act. Indeed, I was told that it was of much value in bringing home to young research workers their responsibilities to experimental animals. Neither the individual licences nor the pain conditions under which they worked were burdensome. The general attitude was summed up later, when Sir Graham Wilson and Dr Lawrence Abel came to the United States to testify at a Congressional hearing on 30 September 1965. Dr Abel, a former vice-president of the Royal College of Surgeons, said in part: "We do not commit the atrocities which are reported from time to time in some other countries. We do not allow the extravagant cruelty committed by some investigators of stress and shock. We have proved that the desired results can be obtained by less inhumane methods". This testimony was so telling that the sub-committee chairman, to whom a word from NIH was law, decided to suppress it. The second day of the hearing was cancelled, and the hearing record was never published.

I returned from Britain convinced that the British act was a boon to animals, admired by British scientists and altogether most valuable despite its age and the need, in certain respects, for updating.

But on the other side of the Atlantic, there was a violent reaction by groups such as the National Society for Medical Research when a streamlined bill built on the principles of the British act was introduced by 13 leading senators. NIH's public

response was less frenetic but equally adamant. NIH even went so far as to withdraw and secrete hundreds of copies of a substantial study it had contracted for because the report clearly showed widespread mistreatment of experimental animals and indicated a need for regulatory legislation.

## Blocking tactics

Senator Lister Hill, named after the illustrious British bacteriologist, was NIH's man in the Senate, and he loyally sat on the bill for six years, refusing all attempts to get it to hearings in the committee he chaired. Not until a stolen dalmatian, trucked across state lines by a dealer in laboratory dogs, ignited an unquenchable public interest, did legislation on laboratory animals reach the Senate floor via a different committee. As soon as it did, the vote to protect the animals was unanimous. Unfortunately, the bill (although opposed as fiercely as the better drafted bill based on the British Act) was weak and narrow in its coverage. Amendments passed in 1970 and 1976 finally gave the Secretary of Agriculture authority to include all warm-blooded animals, and required users to report painful experiments and tests conducted without anaesthetic, analgesic or tranquillizing drugs.

Mistreatment of laboratory animals in the United States in some cases involves a failure to provide even basic care, let alone pain-relieving drugs. For example, at the Veterans Administration (VA) Hospital connected with Stanford University Medical School, a medical student working late was startled to find an experimental dog collapsed at the door of his room. He promptly called the VA veterinarian, but she refused to come to treat the obviously suffering animal, so the student took the dog to the veterinarian on duty at the emergency service, who determined that it was past any possible treatment and euthanized the animal. A necropsy showed nothing but hair in the animal's stomach. The festering sores showed no sign of treatment. The hospital's claims that nothing was seriously wrong and that another dog had inflicted the injuries were hopelessly unconvincing.

Refusal to acknowledge error, no matter how grossly evident, appears to be a common weakness among those who neglect and/or mistreat laboratory animals.

The veterinary inspectors of the US Department of Agriculture (USDA) sometimes try to use their limited authority under existing law to require proper procedures. For example, at the University of Chicago, brain surgery was being conducted on squirrel monkeys in an office off a busy corridor. An office table was used as an operating table. Instruments were not properly sterilized. The inspector called for immediate transfer to one of the university's regular operating rooms.

The proliferation of poorly controlled experimentation in dozens of sites on a

campus makes USDA's task difficult, and since the financial provision for enforcement of the Animal Welfare Act has never been adequate, it has sometimes been years before every nook and cranny where animals are stowed away has been ferreted out. At the University of California at Berkeley, it took a new inspector to find them all. USDA finally brought a case against the Berkeley campus, charging major violations. The case was recently settled when the university agreed in a "consent decision" to pay a \$12,000 fine, establish a training programme for animal handlers and to "cease and desist" from violating the Animal Welfare Act.

The seeming inability of this institution to control its faculty and students is revealing in the light of the thick report of its own specially constituted committee emphasizing that "more diligence on the part of the investigators and better discipline of the caretakers at a minimum of additional costs" would resolve most of the problems. A typical USDA inspection report reads: "Monkeys in room G25 are fed by placing feed in waste pan under floor grill — no feeders present . . . in many rooms a build-up of faeces . . . no regular observation of animals by caretaker under veterinarian's supervision. Some question of DVM [Doctor of Veterinary Medicine] even having access to animal quarters let alone establish an adequate programme of veterinary care."

Reports for all 1,166 Registered Research Facilities in the United States may be obtained under the Freedom of Information Act (FOIA); the Animal Welfare Institute [of which the author is president] has been studying a series of these over the past six months. The reports are astonishing — Harvard, Yale, Johns Hopkins, Vanderbilt, the universities of Utah, Rochester, Pittsburgh, each receiving between \$17 and \$51 million a year from NIH, all accredited by the American Association for the Accreditation of Laboratory Animal Care, regarded by NIH as an ironclad guarantee of first-class care and treatment of animals, repeatedly violate USDA's minimum standards.

Most frequently recurring are: cages too small for monkeys, dogs and rabbits to make normal postural adjustments, green scum in the water bottles, inadequate ventilation, and just plain filth. These notes on the University of Utah are typical: "Primates have inadequate space . . . filters are pretty well plugged with hair . . . large rabbits [weighing] 9 to 11 pounds have 432 square inches and they should have at least 540 . . . some of the cages are not high enough that rabbits can stand up . . . feed in some feed containers contaminated with urine from top cage." Or from Rochester: "Primates in Room 6-7573B are being kept in cages which have not been sanitized for over 6 weeks." At Pittsburgh: "The [cat] room did not appear to have been cleaned since the last

inspection . . . 24 hours for correction." At Harvard a list of 23 different animal rooms housing dogs, cats, guinea pigs, rabbits, gerbils and monkeys used old, rusty, difficult-to-clean cages. The inspector also noted "loose sharp wires" and wrote "it appears sanitation is non-existent while animals are in isolation chambers". One might suppose that correction would be automatic when USDA inspectors write such comments and the institution's attending veterinarian signs off on them. But unless the inspector actually recommends that USDA's General Counsel prepare a case against the institution, the deficiencies frequently drag on, as documented in the Animal Welfare Institute's files.

The USDA inspectors urgently need the increased authority that the Dole and Brown bills would give them, and they need the assistance which institutional committees under this legislation would provide. Key provisions include the appointment of a committee member not employed by the institution "responsible for representing community concerns regarding the welfare of animal subjects", and the requirement that the committee make semi-annual inspections of "all animal study areas and facilities". In addition institutions would be required to inform their employees to report violations of the law to the institutional committee so that prompt corrective action can be taken. Employees would be protected from discrimination against them which many fear at present if they request better treatment of animals.

The basic purpose of the Dole and Brown bills is to prevent needless suffering before it occurs. By putting all personnel on notice that they are not to turn a blind eye to such suffering, the observant and compassionate would occupy the position now frequently usurped by the callous and domineering. All too often, when I visit laboratories I hear the words, "but don't say I told you". This is absurd when the heads of institutions urgently need to know about mistreatment of animals. If they do not know and thus cannot take effective corrective action, they are increasingly likely to find themselves in the embarrassing position that scandals nobody dared to tell them about are publicly revealed. The consequence of that will be increasing erosion of public faith in institutions supported by the tax payers.

Prevention of needless suffering would also be advanced by the provisions of the Dole/Brown bills that aim to eliminate the wasteful duplication of animal experiments and tests. Unintended duplication of government tests was estimated in 1981, by the head of the Interagency Regulatory Liaison Group, to have wasted thousands of millions of tax dollars, yet there is nothing in the Animal Welfare Act as it stands to prevent recurrence of this needless use of animals. The Dole/Brown bills would establish an



information service, in cooperation with the National Library of Medicine, to prevent such duplication. (Purposeful replication of research would be unaffected.) This service would also handle information on alternatives to animal experimentation and testing.

Enlightened self-interest alone should cause every medical school to back the Dole and Brown bills. Failure to protect an investment of millions of dollars by supporting reasonable legislation is shortsighted. But such is the prejudice against legislation, especially on the part of those who do not read the words but rely on lobbying organizations' generalizations, that the Dole and Brown bills are still widely resisted. Organizations that fought the 1966 Laboratory Animal Welfare Act with almost hysterical intensity now, having become accustomed to it, join in testimony before Congress to increase support for its enforcement. No doubt they will do the same if the pending amendments to improve the law are passed.

But the opposition is stepping up its activities. At least that is the sinister reading of the recent decision by the executive director of the Association for Biomedical Research (ABR) to move to Washington, DC. ABR was founded in 1979 by a small group of animal breeders (including Henry Foster, founder of Charles River Breeding Laboratories, the largest purveyor of laboratory animals in the world) and biomedical researchers who felt a need for "active representation" on legislation affecting the use of animals. The organization includes about 200 institutions, including medical schools, pharmaceutical companies and breeders, who depend on animal research. Anyone who doubts that animal research is big business should examine the case of Charles River, which began as a two-room rat-breeding facility and which was recently acquired by Bausch and Lomb, the optical company, in a stock transfer that netted Foster nearly \$38 million, according to press accounts.

The spectacular growth of the industry might be slowed by the Dole/Brown bills' requirement that investigators consider alternatives to animals, and by the information service that the bills would establish to provide facts on "methods which could reduce or replace animal use".

Not surprisingly, ABR has favoured substitute legislation sponsored by Senator Orrin Hatch (Republican, Utah) and Senator Edward Kennedy (Democrat, Massachusetts) that would call on the National Academy of Sciences to conduct an 18-month study of the current use of animals in research. This legislation has already been passed by the House of Representatives, incorporated as part of the NIH authorization bill.

Although ABR says that a study is needed in order to determine whether problems really do exist in the use of research animals, its director, Frankie

Trull, offered a possibly more candid explanation of its support for study legislation in a recent talk she gave to faculty, staff and students at the University of Illinois at Chicago Health Sciences Center. Trull told the audience: "Now, we are criticized for being strong proponents of study legislation by animal welfare organizations who say this is a stalling tactic. Well . . . none of us was born yesterday. The fact of the matter is that, in some ways, it is a stalling tactic." And in response to a question from the audience on how to deal with the media, Trull offered this advice: "You don't answer their questions. In other words, they'll say, 'isn't it true that 83 per cent of all animals had pain-killing drugs withheld during experimentation in your facility last year?' By the way, they know all that stuff. God bless the Freedom of Information Act, they know everything about everything. What you do is you say something like, 'In 1983, in our research institution, we were able to develop a breakthrough in Alzheimer's disease.'" Trull warned that unless researchers take the fight seriously, what happened in Britain could happen in the United States. "England has had horrendous problems, historically, with antivivisectionists . . . there is no question in my mind that the English, in fact, really truly do like their animals better than their people."

In calling on her scientific audiences to do battle with "the other side", Ms Trull deliberately fosters confusion between soundly based regulatory legislation and antivivisectionist sentiment. She emphasizes the rash of break-ins by animal rights groups. "The reason I say please clean up your own shops is that the break-ins are inside jobs, every one of them. By inside jobs, I mean that some sincere, genuine animal technician or cage cleaner or whatever, goes to an animal rights rally or reads an article in a magazine and wants to help the other side. Every single one of the break-ins in the 30 or 40 we're aware of involve inside jobs."

A rational response to this would be to enact the appropriate legal escape valve in the Dole/Brown bills so that personnel who observe mistreatment of animals are protected against reprisals when they blow the whistle — and that a local institutional animal care committee exists to receive such complaints, investigate, and order the problem corrected. But such a sensible procedure is anathema to ABR, which thrives on an "us and them" mentality.

Edmund Burke's advice to choose early reform, which he described as "accommodation with a friend", as against late reform or "capitulation to an enemy," is lost on the lobbyists who fight against proper regulation of animal experimentation. Thus the biomedical community, which needs the Dole/Brown bills as much as the animals do, is listening to badly biased advice inspired by vested interests that seek to increase commercial

profits on animals, equipment and services.

## Pain clause

Most European countries have legislation which addresses pain prevention, often more specifically and strictly than the Dole/Brown bills. The pain conditions in the British act, for example, which have proved workable over more than a century, have spared great pain to large numbers of animals. The proposed bills require assurances that paralytics may not be used without anaesthesia, and pain relief or euthanasia may not be withheld when not scientifically necessary, much milder provisions than those which obtain in Britain.

At present, it is common for animals to be left to die in agony, and no law requires that they be euthanized even when the experiment is over. Nor does current law specify that dogs must be released for exercise outside their cages. The Mayo Clinic is still hosing cages with the dogs inside. Small wonder that contagion is rife among the more than 500 dogs recently viewed by the 13 owners of stolen dogs that Mayo had purchased, despite repeated warnings that many of the dogs they kept buying seemed to be pets. Before the recent scandal broke, Mayo even bought dogs at the laboratory door. Now a sign is posted: "All please note. We no longer purchase dogs from individuals. But we continue to take them as gifts." It was through this unexpected review of Mayo practices that the dehydration of Mayo dogs came to light. Two owners reported that their dogs immediately drank five-quart pails of water on their return home, so intense was their thirst.

It is only natural for people to become angry when the privilege of using laboratory animals is so grossly abused. The US Congress and people generally want the abuses stopped. Neither Congress nor the overwhelming majority of the public want a ban on experimentation. Scientists of good will should recognize this, reject the paranoia sown by lobbyists and join with legislators such as George Brown, a tried and true friend of science, and Senator Dole, whose record on fiscal responsibility has earned him great respect for sound decision making.

"Those who experiment upon animals", Albert Schweitzer wrote, "by surgery and drugs or inoculate them with diseases in order to be able to help mankind by the results of pain, should never quiet their consciences with the conviction that their cruel action may in general have a worthy purpose. In every single instance, they must consider whether it is really necessary to demand of an animal this sacrifice for men. And they must take anxious care that pain be mitigated as far as possible." □

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# New ways with solar neutrinos

*The discrepancy between predicted and measured neutrinos from the Sun is an embarrassment. Now there are two schemes for improving on past measurements. They deserve serious backing.*

MUCH of the present excitement in cosmology rests on the recognition that there must be a close connection between this conceptually esoteric field and high-energy particle physics, the stamping-ground of those who would push experimental measurement to the limit. The result is that people are always speaking in measured tones about the common ground between the very large and the very small.

And there are successes on the record. Astrophysics shows the neighbourhood of a neutron star to be a kind of natural high-energy physics laboratory. The abundance of deuterium relative to hydrogen in the Universe now is almost exactly that calculated from the assumption that the big bang was a time when particles now unstable were predominant in the Universe. And the temperature of the microwave background, 2.7 K, while it is explicable in other ways, is consistent with the view that the whole world was once a high-energy physics free-for-all. So is it not at least a minor scandal that there persists such uncertainty about the Sun's output of neutrinos? Especially when the energies supposedly carried by those particles are measured in MeV, not the GeV which are now the common coinage of the particle physicists?

It is now more than a decade since R. Davis Jr reported the first results with his unique neutrino detector, essentially a huge tank containing 380 cubic metres of tetrachloroethylene buried deep in the Homestake Mine in Colorado. The simple objective was to count the number of chlorine-37 nuclei converted into argon-37 by interaction with a neutrino (and the subsequent loss of a negative electron). The difficulty, ten years ago, was that the rate of conversion and thus the inferred intensity of the neutrino flux was almost an order of magnitude less than expected from what is known of the nuclear reactions in the Sun. All this is now part of the general lore.

Not much has happened since. Or, rather, there has been much talk and speculation but very little experiment. For a time, there was some hope that the discrepancy might be explained away on the basis of the experiments carried out by F. W. Reines at the University of California, Irvine, whose reports of neutrino "oscillations", the quantum mixing together of different types of neutrinos to form the neutrinos of the real world, also bore on the possibility that

neutrinos may have a non-zero mass.

But that line of thought has become unfashionable. The experiments are not easy to repeat, and in any case the cosmologists no longer hope to close the Universe with neutrinos of small mass (a fraction of that of the electron). And while the Davis measurement may be reconciled with expectation by using extremes of several astrophysical parameters, leading in particular to a low core temperature, that seems an ungainly way out of the difficulty.

So what is there left to do? Simply elaborating the Davis measurement would be unprofitable, with a rate of conversion of chlorine into argon only barely above the background. J. Bahcall at Princeton has for the past six years been canvassing the use of gallium-71 as a detector isotope. He would buy a stock of several tons of gallium metal, bury in a mine for several years and then sell it back to the original owners. That device would have the virtue of being sensitive to the least energetic and most abundant neutrinos from the Sun, those produced by the direct interaction of protons. One of these fiscal years, the US Congress may look kindly at the project.

Meanwhile Davis (at the Brookhaven National Laboratory) and a group of colleagues scattered over the United States have suggested yet another neutrino detector. The principle is again the same — convert an isotope of one element into an isotope of another by neutrino interaction, making sure that scores or hundreds of atoms of the product can be measured. The new plan is to convert bromine-81 into krypton-81 (again by the absorption of a neutrino and the loss of an electron). But this time, the product is only weakly radioactive (with a half-life of 200,000 years).

In principle, the experiment (if ever carried out) would have the advantage of being neatly complementary to that proposed by Bahcall. Indeed, bromine-81 nuclei would interact only with neutrinos whose energy exceeded the threshold of 470 keV, which implies that they would be converted into krypton nuclei chiefly by neutrinos arising within the Sun from the radioactive decay of boron-7.

The hope that the quantities of krypton-81 produced can be measured will stretch most people's credulity. If the calculations are correct, krypton isotope atoms should accumulate in the 380 cubic metres of the tank (supposed filled with an organic compound of bromine) at a rate of about two a day, or some hundreds every

six months. How are such small quantities of a stable isotope to be detected?

Obviously it helps that krypton is a rare gas, essentially insoluble in organic liquids. Indeed, using their tank filled with its organochlorine liquid, Davis and his group have been able to test the feasibility of what they propose. Having a system for the extraction of argon from the tank, they can just as easily isolate krypton. They find that leakage of atmospheric krypton into the system should not be a serious source of whatever krypton-81 might be produced by neutrino conversion. After say a year, their bromine tank might contain 1,000 atoms of artificial krypton-81.

But how to measure such tiny amounts? Perhaps because they are anxious not to discourage potential backers, the authors make light of the difficulties in their account of a simulation they have carried out (G.S. Hurst *et al. Phys. Rev. Lett.* **53**, 1116; 1984).

The technique consists merely of repetitive mass spectroscopy. Atoms of krypton-81 in what is supposed to be a mixture of krypton isotopes are selectively ionized by a suitable laser and then accelerated selectively and embedded in a silicon target. Since the ionization process will not have been entirely selective, the embedded atoms can be recovered by heating (again with a laser) and put through the process a second time. And, if necessary, a third. Finally, the atoms are embedded in a copper/beryllium target.

Whatever the likelihood that the technique will eventually settle the problem of solar neutrinos, it seems certain to be used widely in other connections. What the authors show, however, is that starting with a sample containing 1,000 atoms of krypton-81 and the contamination expected in a neutrino experiment, they should be able to count as few as 300 atoms. Laser improvements already foreseen should reduce the limit to about 100 atoms.

For many people, this would be sufficient. But plainly Davis and his colleagues intend a renewed assault on the measurement of solar neutrinos. Indeed, they talk of the design of their complementary detectors as the first steps in the spectroscopy of solar neutrinos. They should be given a chance to put their scheme to work and save the nexus of cosmology and physics from the embarrassment on its doorstep.

**John Maddox**



## Atmospheric chemistry

## Implications of Arctic air pollution

from S.A. Penkett

In winter, the southward movement of the polar front allows air from polluted regions of the Northern Hemisphere to be transported into the Arctic, giving rise to a phenomenon known as 'Arctic haze'. This can lead to a visibility in this pristine area of the Earth's atmosphere of as low as 5 km. The scientific community has been aware of the phenomenon for several decades but a detailed study has been impeded by the remoteness and inhospitable nature of the region. Recently, a series of 28 papers has been published (*Geophys. Res. Lett.* 5; 1984), describing experimental data obtained in a three-dimensional coverage of the atmosphere from Alaska to northern Norway. The papers deal with the meteorology that causes the phenomenon, and with many other aspects which have relevance to such topical concerns as the long-range transport of acidity and climatic change caused by anthropogenic activity.

The results mostly come from the Arctic Gas and Aerosol Sampling Program. It involved the use of six aircraft flying over the Arctic, collecting information on the nature and concentration of the particles making up the haze and on the composition of the associated air. The programme was organized by Russell C. Schnell from the University of Colorado. Scientists came from the United States and Europe, with the majority of the Europeans coming from the Norwegian Institute for Air Research. Funds were provided by the National Oceanic and Atmospheric Administration and by the British Petroleum Company. It is an example of the sort of cooperative programme that has to be mounted if air pollution problems of regional or global significance are to be tackled in detail.

Previous studies at ground level had shown that the aerosol consists mainly of fine particles in a relatively narrow size range (0.2–0.4  $\mu\text{m}$ ). Its major constituent is sulphate, but because of the presence of substantial amounts of black carbon, it has been referred to as a 'combustion' aerosol. The major source areas are Europe and north-west Asia and the intensity is greatest in March and April. The aircraft surveys took place during this period and showed the presence of a complex layered structure, with well defined 'fronts' dividing clean and polluted air. The structural discontinuities are very similar to those found at the boundaries of cirrus clouds, suggesting high thermal stability and lack of turbulence throughout much of the Arctic atmosphere in winter conditions.

The origin of the fine particulate is thought to be anthropogenic but new evidence is presented of the possible volcanic origin of the giant ( $> 2 \mu\text{m}$  diameter) particles. These are spread uniformly at latitudes from 0.3 km to 5 km and are not correlated with the haze layers.

Much of the sulphate in the haze layers is in the form of sulphuric acid and by contrast with other anthropogenic aerosols collected in urban areas of Europe and the United States, there is very little nitrate. The same is also true of the composition of snow samples collected in Spitzbergen.

The low abundance of nitrate is very intriguing. It could indicate that the aerosol is directly injected from combustion processes in the source regions and, indeed, evidence is presented to show that during the Arctic winter, particulate sulphate is not formed by atmospheric gas-to-particle conversion on any significant scale. (That is perhaps not unexpected because of the almost complete absence of any atmospheric photochemistry in the region during the winter months.) A photochemical process may be possible, however, in the source regions at lower latitudes, in March and April. A thorough chemical study of Arctic air pollution may shed light on acid formation in the atmosphere in general. Specifically, measurements of nitrogen oxides and peroxyacetyl nitrate, as well as sulphur dioxide, would define the emission characteristics of the source region and the nature of the chemical reactivity of its atmosphere in early spring and late winter.

The geographical origin of the air carrying the haze components has been investigated in several ways. One is by analysing the distribution of trace metals. High concentrations of elements such as nickel, lead and zinc may be traceable to smelters at particular locations deep inside the Soviet Union.

Another specific source of particulate material was identified in aircraft flights across a fold in the tropopause: volcanic debris was found to be entering the troposphere from the stratosphere in March 1983, presumably from the eruption of El Chichón in 1982. Considerable evidence of the influence of volcanoes on the particulate content of the Arctic atmosphere is stored in ice cores from Greenland. By contrast with the measurements reported for Spitzbergen, the Greenland ice core data suggest the presence of summer maxima in nitrate concentrations. Extensive studies of the composition of the ice in different parts of the Arctic could provide interesting details

on the distribution of source areas.

Measurements of many gaseous species, including sulphur dioxide, ozone, halocarbons and hydrocarbons, are reported. Aircraft measurements show rather small fluctuations in the ozone vertical profiles, and no large changes within or outside the haze layers. The sulphur dioxide concentration peaks in phase with the aerosol in March–April. Large seasonal variations in the concentrations of many other gases were found. In winter, the Soviet Union seems to be a particularly abundant source of hydrocarbons, with the result that the concentrations of propane and butane are as high in March on Spitzbergen, as in air close to London in August. If these values are representative of the whole of the Arctic region, huge reservoirs of these gases could be built up during the winter to be consumed by tropospheric chemistry at lower altitudes as meteorological and sunlight conditions allow. This would probably lead to the production of large amounts of ozone in the troposphere of the Northern Hemisphere and may account for some of the interhemispheric asymmetry observed for this important trace gas.

The profusion of measurable trace gases, including five distinct bromine-containing species, and the absence of photochemical change in the Arctic winter, may allow a specific categorization of air mass sources. It is not unlikely that their relative concentrations will act as a 'fingerprint' for a source area as with no change in distribution during transport, the 'fingerprint' will remain intact over air-mass tracks of many thousands of miles. A detailed examination could form the basis of an effective large-scale tracer experiment.

Many papers deal with the climatological consequences of the Arctic haze. The planetary albedo can be changed significantly by modification of light scattering, such that an increase of 2.5 per cent over the oceans and a decrease of 9 per cent over the icecap is predicted. The presence of graphite particles in the haze layers (at concentrations within a factor of 2 of those recorded in some cities in the United States) has a substantial effect on the optical depth of the atmosphere and is predicted to increase the energy absorbed by the Earth-atmosphere system by as much as 5 per cent. The climatic effects of aerosols of this type, injected into the atmosphere as a result of a large-scale nuclear exchange, are of much current interest. Fortunately, the Arctic haze phenomenon is on a much smaller scale, but studying it may provide quantitative data which can be used to test predictions of the temperature changes likely to be experienced in the aftermath of a large-scale nuclear war. □

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## Malaria

## Blood-stage antigens cloned

from K. N. Brown

THE development of DNA cloning and bacterial expression techniques has opened up a new approach to studying the antigenicity of malaria parasite and, perhaps, to developing a malaria vaccine. Five groups<sup>1-5</sup> have recently achieved expression of cloned genes encoding sporozoite and blood-stage proteins of the human malaria parasite (*Plasmodium falciparum*). Intriguingly, four of the antigens contain short repetitive sequences. The significance of these sequences is not known, but they may have a role in generating antigenic diversity and preventing host immunity.

Two papers<sup>1,2</sup> were discussed recently in these columns<sup>6</sup>. Of the others, Coppel *et al.*<sup>3</sup> have cloned an antigen associated with the membrane of infected erythrocytes. Immunoprecipitation and immunoblotting identified the antigen as a 155,000-molecular-weight (155K) acidic protein although some sera also reacted with a 210K polypeptide. The smaller molecule is found predominantly in the immature 'ring'-stage parasites and the 210K antigen in mature parasites. This may indicate a precursor-product relationship, particularly since the mRNA appears to be abundant only in fully mature parasites. Presumably the 155K portion is carried into erythrocytes by invading merozoites. Sequence data show a subunit structure of five repeats encoding Glu-Glu-Asn-Val-Glu-His-Asp-Ala. Immediately C-terminal are repeats of Glu-Glu-Asn-Val codons with Glu-Glu-Val interspersed irregularly. There is so far no evidence that antibody reacting with the membrane antigen of ring-infected cells is protective.

Another gene encoding a repetitive sequence, in this case one of nine amino acids, Glu-Glu-Val-Val-Glu-Glu-Val-Pro, and associated with the membrane of erythrocytes infected with mature parasites, has been isolated by Koenen *et al.*<sup>4</sup> (see page 382 of this issue). Early work showed that erythrocytes infected with mature trophozoites and schizonts had exposed antigens. Two of the antigens on schizont-infected cells have been characterized: an antigenically diverse polypeptide possibly associated with the 'knobs' of *P. falciparum*-infected cells<sup>7</sup> and the phenotypically varying SICA antigen of *P. knowlesi*<sup>8</sup>. Whether the sequence identified by Koenen *et al.* relates to either of these polypeptides, and indeed how these two types of antigen are related, remains to be determined.

Of the cloned antigens of asexual erythrocytic parasites, the only ones known from *in vivo* evidence to have some significance for protective immunity belong to a family of polypeptides occurring in all

species of mammalian parasite so far examined and varying in size from 190K to 250K. The effectiveness of protection induced varies greatly with the host-parasite combination used<sup>5,9</sup>. In this issue of *Nature* (page 379), Hall *et al.* report that vaccination of Saimiri monkeys with one member of this family, the *P. falciparum* p190 antigen, provides slight protection<sup>5</sup>. This antigen shows extensive diversity between isolates, although there are common epitopes present on the molecule, and many different serotypes can be isolated from one patient<sup>10</sup>. The antigen, or a processed part of it, is present on the merozoite. Hall *et al.* have cloned what is probably its C-terminal end. The sequence is non-repetitive, but it would be surprising if repetitive sequences did not occur elsewhere in the molecule since merozoites and sporozoites are similar in ultrastructure and in function, both being designed to penetrate cells, and the sporozoite surface antigen has well characterized repeat sequences.

Extensive phenotypic variation during chronic infection has been demonstrated for several surface-exposed parasite antigens, including possibly the 190-250K family. It may well be that variable sequences are inserted into all the exposed antigens of malaria parasites, which would provide a solution to two problems: restriction of numbers to ensure host survival and continued parasite survival for vector transmission. Phenotypic antigenic variability is seen in protozoa other than the malaria parasite. The ciliate *Paramecium* is a classic example of a free-living protozoan which adapts its surface macromolecules to environmental change; the changes are readily detectable serologically and indeed can be triggered by antibody. It seems likely that this immunologically important variation results from a general capacity for genetic rearrangement found throughout the protozoa which is exemplified strikingly in the variant surface glycoprotein genes of *Trypanosoma brucei* and has recently been inferred in other parasites<sup>11-13</sup>. In the case of malaria parasite, some of the diversity detected serologically, whether genotypic or phenotypic, may not necessarily be a response to immune pressure, but rather may reflect the requirement of an obligate parasite to enter and survive in genetically diverse hosts.

If genetic diversity and rearrangement are part and parcel of malaria parasites, what hope is there for a malaria vaccine? Given the epidemiology of malaria it is hardly surprising that with the exception of a few highly-selected laboratory systems, immunization with crude or purified anti-

gen is relatively ineffective. Protection has not so far been achieved with the sporozoite surface antigen — is this a result of antigenic diversity<sup>6</sup>, immune evasion by antigen capping or inappropriate vaccination technique? Perhaps immunization with vaccinia virus-containing sporozoite sequences<sup>14</sup> will answer the last question. The requirement for draconian adjuvants and the antigenic diversity of the merozoite surface antigen do not encourage much hope for protective cross-reactivity through common epitopes, although there is preliminary evidence that isolates can be ranked for cross-protective activity. Good immunity to virulent blood-stage infection can, however, be obtained using avirulent K<sup>-</sup> *P. falciparum* lines<sup>15</sup> and  $\gamma$ -irradiated non-replicating parasitized cells without adjuvant. These results may indicate that parasite antigen seen in the context of modified erythrocyte is the required immunogen. Could such an immunogen be produced in quantity? Does the report of the insertion of new genetic material into haematopoietic stem cells<sup>16</sup> at last begin to approach the level of technical sophistication which immunization against malaria parasite requires, leading ultimately to *in vitro*-grown erythrocytes derived from precursors containing parasite-antigen-encoding inserts in their DNA?

In order to exploit the developments in the molecular biology, a reliable assay of the protective immunogenicity of and responses elicited by the various forms of malaria parasite immunogens *in vivo* will be essential. This is a difficult problem, however, as protective immunity is rarely complete; for example, around 50 per cent of immune adults in holoendemic areas may have parasites detectable in their blood at any one time, and recent or current low-level parasitaemia may be a prerequisite for preventing clinically significant infection. Many attacks of malaria over several years are required to generate clinical immunity, and immunity is in part strain specific. The diversity of malaria parasites and the complexity of the epidemiology also make the interpretation in terms of protective immunity of the new epitope and sequence data on surface antigens extremely speculative at this stage. □

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## Gene regulation

## A step closer to the principles of eukaryotic transcriptional control

from Tim Reudelhuber

In prokaryotes interaction between the genome and sequence-specific DNA-binding proteins has been shown to play an essential part in the control of gene expression. In eukaryotes such a relationship has been much more difficult to prove although evidence is accumulating that certain transcription factors interact with specific promoter elements (see, for example, refs 1, 2). Furthermore, local perturbation of chromatin structure has often been seen over the 5'-flanking sequences of actively transcribed genes. This 'open' chromatin conformation is typified by regions of greatly increased accessibility to cleavage by endonucleases such as DNase I (DNase I hypersensitive sites). These regions have been attracting increasing attention of late, both because they may mark important control regions for gene activity and because we seem to be on the verge of identifying components involved in their generation and, possibly, their function (for an overview of recent advances see ref. 3). To date, the most common finding has been that DNase I hypersensitive sites are missing in the promoter of 'silent' genes and become apparent in tissues which are either expressing or have the potential to express the gene in question. Two weeks ago, Fritton *et al.*<sup>4</sup> reported in *Nature* an interesting new twist to this now familiar story, for the DNase I hypersensitive sites flanking the chicken lysozyme gene.

In the chicken, the gene encoding lysozyme is expressed constitutively at low levels in mature macrophages, and is expressed at high levels in response to steroid hormones in the oviduct. The gene is not expressed in the embryo or in such tissues as brain, liver, kidney and erythrocytes. Fritton *et al.* found that in all the non-expressing tissues studied, with the exception of erythrocytes, there was only one DNase I hypersensitive site (which I shall refer to as B; the other hypersensitive sites are named A, C-E to simplify discussion) upstream of the startsite of transcription. In erythrocytes no hypersensitive sites were detected. In the constitutively expressing macrophages, sites A, D and E were detected while in the hormone-stimulated oviduct an alternate set of sites, B, C and E, was present; site C disappeared when hormone stimulation was withdrawn. What these data suggest is that it is no longer appropriate to refer to the active chromatin conformation of an expressed gene, since different tissues may manipulate the promoter elements in different ways to either 'turn on' or 'turn off' a transcription unit.

An obvious benefit of such a thorough cataloguing of the hypersensitive sites and their correlation with the transcriptional activity of the gene is that it becomes possible to venture an educated guess as to their function. It is known that active transcriptional enhancer elements are capable of inducing regions of DNase I hypersensitivity<sup>5</sup>. In the mouse mammary tumour virus, where an enhancer has been described whose activity is glucocorticoid dependent, a DNase I hypersensitive site appears transiently over this element only during hormonal stimulation<sup>6</sup>. Since site C in the lysozyme gene behaves in an analogous fashion, this region of the promoter is a good place to search for a similar hormone-modulated enhancer whose activation may explain the surge of lysozyme expression in the oviduct. Using similar logic, site B, which appears in most of the non-expressing tissues (but also in the oviduct), may mark the position of the transcriptional repressor element whose effect is overcome by an interaction at site C in the oviduct. The absence of this site in the non-expressing erythrocyte suggests that the cell may have yet another option at its disposal for suppressing lysozyme gene activity. Finally, sites A and D, seen in the macrophage, could mark the position of an alternative set of control elements capable of achieving low-level constitutive

expression of the gene.

Of course, the actual function of all these sites remains to be demonstrated. A crucial question to answer will be whether the 'open' chromatin structure of these regions *per se* is important to their function, or whether the sites are simply 'footprints' generated by the interaction of regulatory proteins with the promoter elements. In other words, are the chromatin rearrangements at the hypersensitive sites a prerequisite to the binding of regulatory transcription factors or a consequence of it? *In vitro* systems in which both DNase I hypersensitivity and regulated transcription can be reconstituted will ultimately answer this question. Recent progress has been made in identifying erythrocyte-specific factors involved in generating a DNase I hypersensitive site in the chicken  $\beta$ -globin gene promoter<sup>7</sup> and, in a recent issue of *Nature*, Carl Wu described the *in vitro* reconstitution of factors binding in the hypersensitive region of *Drosophila* heat-shock genes<sup>8</sup>. Such advances should take us a step closer to understanding, in molecular terms, the relationship between chromatin structural rearrangements and the control of gene expression at the transcriptional level. □

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## Synthetic metals

## Polyacetylene and organic superconductors lead the field

from Martin R. Bryce and Munir M. Ahmad

A RECENT conference\* on the 'Physics and chemistry of synthetic metals' confirmed the unabated interest of organic chemists, physical chemists and solid-state physicists in this field (see Bryce, M.R. & Murphy, L.C. *Nature* **309**, 119; 1984 for a review). Significant progress in the two central research themes, conducting polymers and conducting single crystals, has been made since the Les Arcs meeting eighteen months ago.

Polyacetylene remains the most popular compound with the physicists. It is becoming increasingly clear that the quality of the material and the precise nature of any impurities are of central importance to a rationalization of solid-state properties. A two-step synthesis of polyacetylene was

described in which a prepolymer is prepared and then purified and cast into films or fibres before conversion to the polymer by elimination (W.J. Feast, University of Durham). This method produces polyacetylene of high purity and overcomes some of the crystallinity and morphology difficulties of the original Shirakawa route. Homogeneous dense films of (CH)<sub>x</sub> can now be prepared and iodine-doping proceeds rapidly to 20 molar per cent to yield material with a conductivity of 10 ( $\Omega$  cm)<sup>-1</sup>. By contrast, AsF<sub>6</sub> doping proceeds only to a level of 14 per cent. Optical, infrared, magnetic and transport properties of these doped films are similar to those measured for Shirakawa polyacetylene. The nature of polarons and solitons, their lifetime and charge storage, were considered for the

\*Held in Abano Terme, Italy, on 17-22 June.

chemically-induced defects in polyacetylene (J. Schrieffer and A. Heeger, University of Santa Barbara).

Several novel polymeric aromatics were discussed. Poly(isothianaphthalene), a transparent organic conductor with a band gap of only 1 eV, attracted special attention (F. Wudl, University of Santa Barbara). Other important polymers were a fluorinated analogue of poly(1,6-heptadiyne), which is an intrinsic semiconductor with enhanced oxidative stability; a new phase of poly-peri-naphthalene; soluble substituted polyacetylenes, such as poly(trimethylsilyl)acetylene, which can be desilylated to a controlled degree to produce a copolymer; and polypyrrole and related polymeric aromatic heterocycles.

Theoretically, there is still controversy over the quantum statistics of fractionally-charged excitations. Schrieffer discussed this topic in the light of proposals that quasi particles of fractional charge are responsible for the fractional Hall effect in low-dimensional materials.

Over the last two years, the field of conducting single crystals has deservedly been dominated by salts of tetramethyltetraselenafulvalene (TMTSF) which are superconducting at very low temperatures and seem to possess many unique remarkable properties. The development of more sophisticated and elaborate theories confirms that no consistent interpretation of this class of conductors can be reached unless one-dimensional physics is used as the starting point (D. Jerome, University of Paris-Sud, Orsay). A clearer understanding of the fascinating phase diagrams is emerging, and for (TMTSF)<sub>2</sub>PF<sub>6</sub> evidence for transitions from metallic to spin-density wave state to superconducting state as temperature is lowered at constant pressure was presented (J. Schirber, University of New Mexico, Albuquerque).

Crystalline salts of bis(ethylenedithio)tetrathiafulvalene (BEDT-TTF) present an exciting new class of materials. (BEDT-TTF)<sub>2</sub>ReO<sub>4</sub> is a high-pressure superconductor. S. Parkin (IBM Labs, San José) correlated the absence of superconductivity in the majority of BEDT-TTF salts with their high dimensionality compared with the 2:1 ReO<sub>4</sub> salt. Applications of single-particle-band theory to the electronic structure of the TMTSF and BEDT-TTF salts reveal striking similarities between the two families; (BEDT-TTF)<sub>2</sub>ReO<sub>4</sub> has the lowest anisotropy of any known superconducting charge-transfer salt.

It is more than ten years since the archetypal organic metal, tetrathiafulvalene-tetracyano-*p*-quinodimethane (TTF-TCNQ), was first prepared and, notably, at the conference this complex was hardly mentioned. There were few reports of new donors. The elusive tetratellurafulvalene derivatives have now been prepared, however. D. Cowan (Johns Hopkins University) described the synthetic approaches and pointed out that although TSeF is

harder to oxidize than TTF, this trend is reversed on passing to TTeF which has an oxidation potential similar to TTF. K. Bechgaard (University of Copenhagen) has synthesized the donor 1,6-dithiopyrene, which forms a complex with TCNQ that, even at ambient pressure, is metallic down to 4 K with no phase transitions.

Electrocrystallization is now a widely-used technique for preparing metallic salts, including radical cation salts of arenes. Intermolecular interactions in salts based on oligomers of poly-*p*-phenylene, containing segments that carry a charge, were considered as structural models for conducting polymers (V. Enkelmann, University of Freiburg).

Linear chain compounds remain a focus for attention. Metallic, as well as semi-conducting, metal-dithiolene complexes are known (A. Underhill and K. Carneiro, Universities of Bangor and Copenhagen respectively), and ferromagnetic compounds comprising ferrocenium and TCNQ units have been characterized by structural, magnetic, spectral and transport data (J. Miller, Dupont Labs).

The industrial future of synthetic metals for technology and applied science seems

bright and there is increasing industrial support. Polyacetylene, polyaniline and poly-*p*-phenylene can be used in batteries and fuel cells employing aqueous electrolytes (A. MacDiarmid, University of Pennsylvania). (An account of recent work on battery performance of polyacetylene by J.C.W. Chien and J.B. Schlenoff appears on page 362 of this issue of *Nature*.) The organic metals that are capable of undergoing phase transitions under optical radiation are being used in electro-optic devices for communication and storage of data (K. Yoshino, University of Osaka) and the first erasable switching device capable of processing information using a single laser source was described (R. Potember, Johns Hopkins University). Moreover, there is potential for organic rectifiers based on a donor molecule linked via an insulating carbon bridge to an acceptor molecule, although because of synthetic difficulties this goal has not so far been achieved. □

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## Molecular biology

# Messenger RNA pieced together

from H.D. Robertson

In a provocative paper on page 350 of this issue of *Nature*, Campbell, Thornton and Boothroyd provide evidence that a short segment at the start of a messenger RNA (mRNA) molecule from a trypanosome can be found as part of a discrete transcript, separate from the remainder of the mRNA. They suggest that either the two segments of the mRNA are transcribed separately and subsequently joined or the shorter RNA segment acts as a sort of primer for transcription of the longer segment. The concrete demonstration of either of these mechanisms would be unique for a non-viral system and, particularly in the case of the priming mechanism, would have very important implications for our understanding of how mRNA synthesis can be regulated.

The expression of variant surface glycoprotein (VSG) genes in the African trypanosome *Trypanosoma brucei* is of extraordinary interest because it is responsible for the successive waves of trypanosomes with different surface glycoproteins that allow the parasite to persist in the face of successive attacks by its host's immune system. Recent work has shown that the mode of expression of these genes — at both the DNA and RNA levels — may reveal new molecular pathways.

Leaving aside for the present discussion the fact that the VSG genes can also undergo DNA-level rearrangements which are related to their expression, it has become

clear that DNA encoding a 35-nucleotide leader sequence (the 'mini-exon') of all VSG mRNAs and that encoding the remainder of VSG mRNAs can be 30 kilobases or more apart. This great separation, found by several groups of investigators including that of P. Borst, coupled with the investigators' inability to find giant mRNA precursor molecules which would be expected in a conventional synthesis/splicing pathway, have led Campbell, Thornton and Boothroyd to seek and characterize smaller transcripts containing the 35-nucleotide 'mini-exon' RNA with the idea that these might somehow become associated with the remainder of the VSG mRNA after their synthesis.

Since the mini-exon RNA itself occurs in many RNAs of various sizes extracted from infected cells, Campbell *et al.* have used sequence information from cloned DNAs containing the mini-exon and its immediately adjacent regions to seek small transcripts. Using restriction fragments of the cloned DNAs together with a synthetic oligonucleotide complementary to a region about 100 bases downstream from the mini-exon DNA sequence, they have applied three standard indirect tests, all of which have independently pointed to the existence of a 137-base RNA species which contains the mini-exon at its 5' end.

Regulation of gene expression at the level of RNA transcription is a phenomenon more widely known at present in



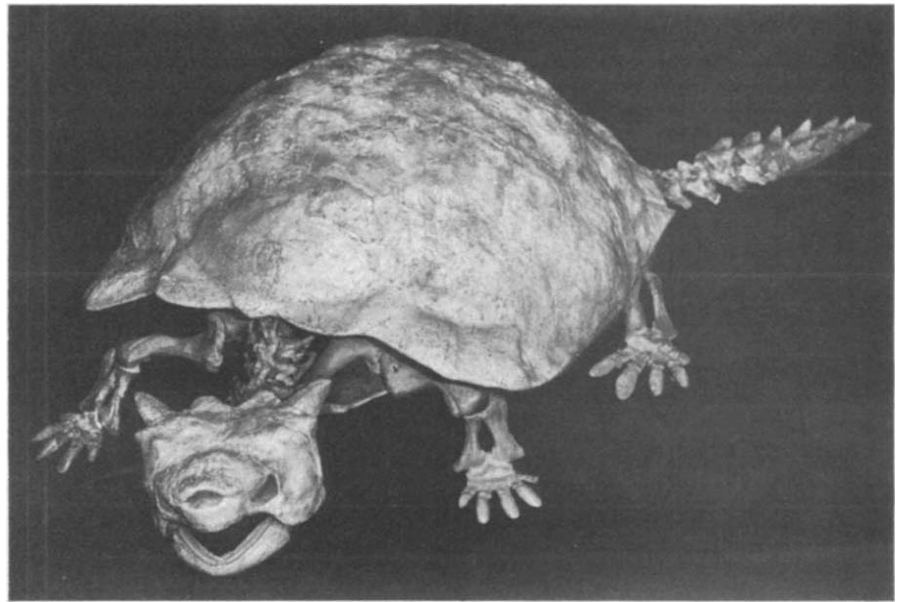
prokaryotes than eukaryotes. In particular, gene regulatory schemes in which a eukaryotic RNA transcribed at one place in the genome is used to mediate RNA synthesis at a second site have been difficult to support with experimental evidence. Except in the case of a few viruses, proposals from the early 1970s invoking the use of a RNA specifically processed from one gene transcript to stimulate transcription at a second genomic site have remained in limbo. One good reason for this state of affairs was the discovery in 1977 of introns in eukaryotic genes, leading to wide acceptance of the existence of an RNA-splicing mechanism to allow the covalent linkage of non-contiguously coded RNA transcripts. Consequently, there seemed little biochemical need for another way to obtain the same result.

Nevertheless, the idea that RNA primers could be cleaved from one RNA molecule and used to stimulate transcription of another remains an attractive hypothesis, supported in recent years by evidence published by R. Krug and his colleagues at the Sloan-Kettering Institute of the use of host-cell RNA primers for influenza virus mRNA synthesis. They could be sure that their data did not reflect a conventional RNA-processing reaction because the primer RNA sequence linked by the 5' terminus of each influenza virus-encoded mRNA segment was demonstrably of cellular origin. Thus, mRNA molecules originating both *in vivo* and *in vitro* could be synthesized in controlled conditions and the origins of their segments analysed in detail.

Until recently, the search for such events — with their regulatory potential — in eukaryotes has awaited a system of sufficient interest to make worthwhile the extra effort needed to distinguish transcription-level effects from those at the level of conventional RNA processing, and in which it was feasible to analyse in detail the expression of various RNA segments comprising a candidate mRNA molecule.

A pool of small RNA molecules containing the mini-exon sequence, such as the 137-base RNA reported by Campbell *et al.*, is a pre-requisite for all models of discontinuous transcription of the VSG genes although its presence does not prove that discontinuous transcription is occurring. Similar results have recently been obtained by Borst and his co-workers using several trypanosome strains (*EMBO J.*, in the press). The next step is clearly the direct analysis of the RNA precursors to VSG mRNA, particularly those containing the mini-exon sequence. In this way, it should be possible to tell whether the RNA segments are made separately and then joined by some unconventional mechanism; whether RNA-primed transcription is responsible; or whether some combination of these events in an even more exotic process could be taking place in this system. □

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## Giant tortoises down under

THE present-day animals of Australia are a peculiar bunch, but those that lived one million years ago, in the Pleistocene, were even stranger. There was a giant wombat called *Diprotodon* which was the size of a rhinoceros, and a monster 3-metre-high kangaroo named *Procoptodon*. This trend to large size was also seen in the turtles. The available specimens of the tortoise *Meiolania* have recently been restudied (Gaffney, E.S. *Bull. Am. Mus. nat. Hist.* 175, 361; 1983) and some important new finds are reported.

*Meiolania platyceps* was a 2-metre-long tank-like land tortoise that is known from numerous remains found on Lord Howe Island, New South Wales. Its skull was heavy and covered with an outer armour of plates and horns — no doubt so that it could withstand the impact of a giant kangaroo landing on its head. *Meiolania*, like most turtles, had a relatively small braincase, so it probably wasn't very bright. Its shell was huge, and its arms and legs could be pulled in beneath it. One remarkable feature of *Meiolania* is its tail which was long and carried at its end a bony mass made from rings and spikes. Like certain armoured dinosaurs (the ankylosaurs), *Meiolania* could have swung its tail from side to side to deliver a powerful blow to any potential predator.

All the material has been collected from shoreline and soil deposits on Lord Howe Island, and these have been tentatively dated as 100,000–120,000 years old. The first *Meiolania* bones may have been collected in 1844, when John Foulis MD visited the island. He later recommended that it be developed as a penal colony, stating that the island could "support a population of 5,000 souls if under control". One wonders what 'control' he had in mind. The island was not developed in that way. Later, Robert D. Fitzgerald visited the island and found turtle bones in

1869. He sent specimens to Sir Richard Owen, the leading British comparative anatomist of his day. Further bones have been collected since then, with recent finds in 1971, 1980 and 1982; most of these went to the Australian Museum, Sydney.

Richard Owen identified the first skull and tail club of *Meiolania* that he saw as that of a giant lizard (1881, 1882) and, later (1886), other remains as those of a large turtle. It was Thomas H. Huxley, a rival of Owen's, who gleefully pointed out that Owen's 'giant lizard' was in fact a turtle (1887). Since then, other British and Australian scientists have identified various *Meiolania* bones, and speculated wildly about the animal's precise taxonomic relationships.

In the new work, Gaffney redescribes the skull in detail, and concludes that *Meiolania* is a cryptodire turtle, related to present-day soft-shell turtles and tortoises.

*Meiolania* died out, with the giant wombats and kangaroos, some time ago. This may have been caused partly by climatic changes during and after the Ice Ages, or by the arrival of humans in Australia. Giant tortoises still survive, but only just, on the islands of Aldabra and the Galapagos, but these are not close relatives of *Meiolania*. *Meiolania* would have been a tempting food-source for humans because of its large size, although it would not have been easy to kill because of the heavy armour over its head and body. It was probably very slow-moving and dim-witted, however, and an enterprising group of aborigines could have stood on the tortoise's back to avoid the tail-club, and attacked its unprotected neck with blunt instruments. □

From Michael J. Benton, who is in the Department of Geology, The Queen's University of Belfast, Belfast BT7 1NN.

## Geophysics

# Continental area and elevation

from A.G. Smith

WHEN all the continents were clustered together as Pangea, a small part of that supercontinent experienced flooding by the sea. By contrast, a much larger area of the smaller continents created by the break-up of Pangea has undergone periodic flooding during global rises in sea level. Because there is no reason to suppose that similar changes in sea level did not take place when Pangea existed, an important aim of geophysicists has been to explain the different flooding frequencies before and after the break-up.

Analyses of the global topography suggest that continental area is correlated with continental height. From this it follows that Pangea, the largest possible continent, would have had the largest average height, thereby reducing the area available to flooding during a global rise in sea level. Two authors<sup>1,2</sup> have recently reassessed the suggested area-height relationship.

On page 370 of this issue of *Nature*, Wyatt<sup>1</sup> discusses some of its implications. In particular, he suggests that the reduction in shelf area that might be expected to have accompanied the formation of Pangea may have been a casual factor in the late Permian extinctions of shelf organisms. Stanley, however, has cited evidence that geologically recent reductions in shelf areas have had little effect on some shallow-water faunas<sup>3</sup>.

In a recent paper Cogley<sup>2</sup> makes a more detailed analysis of continental topography, particularly continental margins, than the analyses by Harrison and others<sup>4,5</sup> on which Wyatt's paper is based. Cogley concludes that modal height — as opposed to average height — is not correlated with continental area.

He cites several areas that are much higher than any height-area relationship based on the major continents would predict, including Arabia and India. The tiny mountainous islands of Corsica and Sardinia in the Mediterranean are also anomalous, though this is not unexpected as they are associated with young mountain-building movements. However, were Madagascar regarded as a distinct continent, it too would be anomalously high. It would seem that the correlation of continental elevation with continental area is not as clear as one might like.

Until one has a working model of what determines the thickness of continental crust and why it should change with area, it is difficult to know whether these exceptions are important. In the case of the oceans, there is an excellent quantitative understanding of the relationship between ocean-floor depth and age<sup>6</sup>. Anomalously shallow ocean floor is simply a place where more molten rock has erupted or been

injected into the crust than normal. Why this should have happened is not clear, but at least the unexpected increase in thickness is explained.

In the case of the continents there is no generally accepted working model of what determines the thickness of their crust. A suggested mechanism of continental uplift is the injection of carbon dioxide and water into the lower crust, but the volumes involved are so huge that such an explanation seems unlikely, except on a local scale.

The only plausible cause is physical. Analogy with the oceans suggests that the most probable mechanism is an increase in heat flow. This could cause uplift simply by bringing about the thermal expansion of the continental lithosphere. Furthermore, the heat could effect crystalline phase changes in the rock minerals, inducing significant volume changes without any change in bulk composition. Uplift associated with uprising convection currents would be unlikely to be as much as the hundreds or thousands of metres observed in, for example, southern Africa. But one can speculate that convection might be more vigorous under large continents and

deliver more heat, and this might partly explain the correlation between area and height.

How can these problems be resolved? An important approach will be to document precisely the changes in continental structure with time. One of the most promising sources of information is the rock fragments brought up from great depths in diamond pipes. It might be possible to infer a continental lithospheric structure from a suite of fragments from an old pipe that is known to have penetrated a continent when it was near sea level and compare it with a suite from a younger pipe that penetrated that continent when it stood at a much higher level. Southern Africa may hold the key to this approach.

It is ironic that our understanding of the continents on which we live is far less advanced than our understanding of the ocean floor, which we rarely see. Discussion of the implications of the correlation between continental area and elevation will help to point the way to changing this state of affairs. □

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## 100 years ago

THE Japanese appear to be determined to render themselves, as far as possible, independent of foreign countries. They have, says the *Pharmaceutical Journal*, established in Tokio a factory for the production of pharmaceutical chemicals on a large scale. A company with a capital of about 40,000l. has been formed for this purpose. Of this amount the Government has contributed one-half free of interest for twenty years, besides making a free grant of land and erecting the necessary buildings. A similar company is taking up the utilisation of the waste *saké* from the native breweries in the manufacture of alcohol, and the manufacture of bleaching-powder on a large scale has been commenced. Whether with the object of "protecting" the first of these enterprises or not does not appear, but we learn from the same authority that an increased tax has been placed in Japan on imported patent medicines, and the nature of the articles to which this has been extended is stated to have largely affected the import of some chemicals into that country. Santonin, which was at one time much in request among the Japanese, decreased 20,000 ounces in import last year, although the price was lower; on the other hand, the consumption of quinine showed and increase.

From *Nature* 30, 512, 25 September 1884.

## A SEA MONSTER

A FRIEND of mine, Capt. W. Hopkins, of the schooner *Mary Ogilvie*, who has just returned from a voyage all round Australia, has given me the following information, which I forward you for publication, not so much because of its interesting character, but in order that other travellers may throw some light upon the character of the animal, which, if an Octopus, must be of much larger dimensions than those usually met with. On June 15, when in S. lat. 21° 37' and E. long. 13° 49', about five miles off the Exmouth Gulf on the western coast of the continent, he saw an immense creature which he took to be a species of Octopus. His attention was drawn to it by a perfect cloud of sea birds, and at first he naturally thought it must be a dead carcass. On approaching it, however, he found it was alive and sluggishly disporting itself. In shape it was like a violin, but of immense size, with some six feelers about the greater diameters of the violin. It lay almost flat upon the water, was of a dark gray above and lighter gray below, and was continually elevating one of its feelers, apparently twice the thickness of a man's arm, to a height of from six to eight feet. It appeared to be vomiting, and as the birds were evidently feeding, that accounted for their presence in such numbers. Its size was so great that, had it grasped the vessel, it could easily have capsized it. The captain therefore got out of the way as quickly as possible, and without making definite measurements; but a large whale in the vicinity looked quite diminutive. It is a pity that something more exact as to size is not available, but I think the description is sufficient to convey an idea of the nature of the monster.



## Immunology

# Receptor gene rearrangement and ontogeny of T lymphocytes

from Miranda Robertson

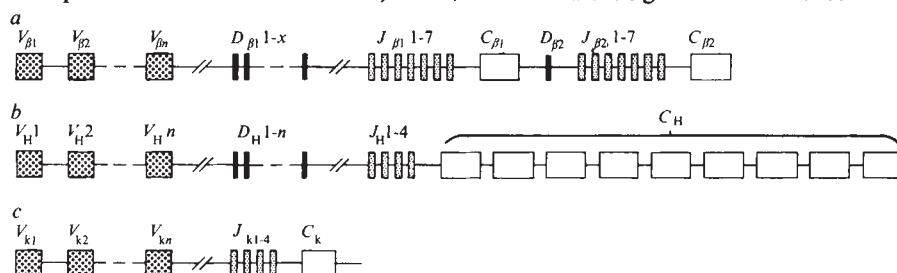
MANY of the outstanding questions raised by modern cellular immunology converge on the issue of T-lymphocyte ontogeny. T lymphocytes, so called because they mature in the thymus gland, provide both the cytotoxic effector lymphocytes that eliminate virus-infected cells, and the helper and suppressor lymphocytes that regulate the activities of effector cells including the B lymphocytes that produce antibodies. The exact nature of the thymic maturation process, however, has proved very hard to define, and even the definition of the different subsets of T cells is not altogether unambiguous. Both questions are in part questions about antigen recognition by T cells.

All T lymphocytes, unlike B lymphocytes, recognize foreign antigen in the context of surface molecules encoded in the major histocompatibility complex (MHC), the cytotoxic cells focusing on the class I molecules and the regulatory T cells on the class II molecules. Do T cells 'learn' to recognize 'self' MHC molecules in the thymus, and during this process acquire both tolerance to self alone and the potential to react to self plus foreign antigen? How might that maturation be reflected in the expression of the genes encoding the antigen receptors and how might that vary between different subsets to account for their differential responsiveness to class I and class II molecules? Five recent papers<sup>1-5</sup>, three of them published in *Nature* this week<sup>2-4</sup>, have

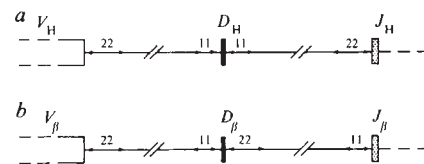
an important bearing on these issues.

All five papers deal with the genomic rearrangements through which the separate DNA segments encoding the  $\beta$  chain of the T-cell receptor are assembled into a complete gene. Since the  $\beta$  chain is only half of the receptor heterodimer<sup>6</sup>, the final answer to the question of antigen recognition will have to await an equivalent analysis of the  $\alpha$ -chain DNA. The present position on the  $\alpha$  chain (see box on page 306) and the mechanism of antigen recognition will be discussed in more detail in a later article in *News and Views*. I shall here confine myself to the limited but important conclusions that can be drawn from the analysis of  $\beta$ -chain gene organization, and its rearrangement in T lymphocytes of different subsets.

It is now clear that the  $\beta$ -chain DNA shows homology to that of the immunoglobulins not only in sequence but also in general organization: Figure 1 shows how the coding segments compare with immunoglobulin gene segments, and the legend contains a brief recapitulation of how complete molecules are generated from the assembled segments. It was already known the last time this topic was discussed in these columns<sup>7</sup> that the T-cell  $\beta$  chain, like the immunoglobulin heavy chain, is assembled from four separate segments:  $V_\beta$ ,  $D_\beta$ ,  $J_\beta$  and  $C_\beta$ . Kavalier *et al.*<sup>1</sup> and Siu *et al.*<sup>2</sup> have since cloned genomic  $D_\beta$  segments and are able to demonstrate a significant difference from



**Fig. 1** Gene segments encoding *a*, the  $\beta$  chain of the T-cell antigen receptor; *b*, the heavy chain of immunoglobulin; *c*, the  $\kappa$  light chain of immunoglobulin. Immunoglobulin molecules are made up of four chains, two identical heavy chains that contain a variable antigen-recognition domain and a constant effector domain, and two identical light chains also comprising a variable and a constant region. The variable regions of the light chains, which can be either of two isotypes, known as  $\kappa$  and  $\lambda$ , are assembled from separately-encoded  $V$  and  $J$  segments that are brought together by genomic rearrangements during the differentiation of the B lymphocytes that produce antibodies. The final mRNA containing the  $VJC$  sequence is produced by processing of the primary transcript from the rearranged gene segments. The rearrangements in the heavy-chain gene pool are more complex, in that three separate segments comprise the DNA encoding the variable region: a  $V$  segment, a  $D$  segment and a  $J$  segment which are sequentially rearranged during B-cell ontogeny to produce a continuous  $VDJ$  DNA sequence. Different combinations of these segments account for much of the diversity of antigen-binding sites in immunoglobulins. The T-cell receptor  $\beta$  chain also contains  $D$  segments, but is organized differently from that of the immunoglobulin heavy chain in that each of the two constant regions has a set of upstream  $D$  and  $J$  segments. Moreover, the two constant regions of the  $\beta$  chain are essentially identical, whereas the immunoglobulin heavy-chain  $C$  regions are quite different and have different effector functions. The final receptor molecule of the T cell has two chains, known as the  $\alpha$  and the  $\beta$  chains; the structure of the  $\alpha$  chain is still problematic (see box).



**Fig. 2** Recombination signals for the immunoglobulin heavy chain (*a*), and for the T-cell antigen receptor  $\beta$  chain (*b*). The separate DNA segments encoding the variable regions of the immunoglobulin genes are flanked by conserved heptamer and nonamer sequences separated by non-conserved spacer DNA that may be either 11 or 22 nucleotides long, representing either one or two turns of the DNA double helix. These conserved sequences are believed to mediate the genetic recombination by which the separate segments are assembled into the complete coding sequence during B-cell ontogeny, according to a rule known as the 11-22 rule whereby recombination takes place only between a conserved sequence pair separated by an 11-nucleotide spacer and one with a 22-nucleotide spacer. Thus  $D-D$  or  $V-J$  joining violates the recombination rules in the heavy-chain gene pool. In the T-cell  $\beta$ -chain gene pool, by contrast, the spacers are so organized that  $D-D$  and  $V-J$  joining would be allowed.

their immunoglobulin counterparts — not in the coding sequence, though that is of course different — but in the flanking DNA that contains the signals believed to mediate their recombination with the  $V$  and  $J$  segments (see Fig. 2). According to the rules that are believed to govern these recombination events (see Fig. 2 legend), neither direct  $V-J$  joining nor  $D-D$  joining is allowed in the immunoglobulin heavy-chain gene pool. In the  $\beta$ -chain pool, however, both are possible without violation of the rules. Such flexibility in the assembly of the gene segments might help to compensate for the apparent absence in the  $\beta$  genes of somatic mutations<sup>8</sup> that are known to contribute to the diversity of the immunoglobulins. The structural consequences of the alternative rearrangements in the T-cell pool might however be expected to be more profound than those of scattered point mutations.

So far, however, there is no evidence either for  $V-J$  or for  $D-D$  joins. What has emerged from more detailed analyses of the structure and expression of the  $D$  segments is an entirely unsuspected feature of T-cell ontogeny that has, moreover, been almost simultaneously discovered to have an exact equivalent in B lymphocytes.

In T cells, as in B-cell heavy-chain genes<sup>9</sup>, the first joining event is between a  $D$  and a  $J$  segment<sup>1</sup>. Siu *et al.* have now discovered that this preliminary joining event results in the production of a  $DJC$  transcript from a conserved promoter upstream of the  $D$  segment. The truncated transcripts, which can involve either of the two  $C_\beta$  segments and its associated  $D$ s and  $J$ s, are extremely common and indeed account for two of the four cDNA clones originally isolated by Hedrick *et al.*<sup>10</sup>. Siu *et al.* report that the truncated transcripts

are prevalent in the thymus and are seen very little in peripheral lymph-node T cells<sup>2</sup>. Their prevalence in the thymus could explain why despite the high levels of T-cell receptor RNA in thymocytes<sup>11</sup>, Kappler was unable to detect corresponding levels of surface protein (see ref. 7).

Otherwise, however, the importance of the short transcripts is a mystery; though important they must certainly be. First, it is clear from a comparison of mouse and human *D* segments<sup>3</sup> that the *D*-segment promoter is highly conserved, and moreover *DJC* transcripts are highly prevalent in man as well, preliminary data suggesting that they are present in large amounts in thymus and small amounts in periphery; and second, Alt *et al.*<sup>12</sup> have recently shown that equivalent *DJC* transcripts are characteristic of immature B cells in which they are transcribed from a conserved promoter and have also been shown to be translated. No one has been prepared to guess at a possible function for the transcripts, but it must be significant that the *D* segment is the most conserved of all the segments; and it may be significant that both mouse and man have maintained duplicate *C<sub>β</sub>* segments that are almost identical.

Since the near-identity of the *C<sub>β1</sub>* and the *C<sub>β2</sub>* segments rules out  $\beta$ -chain constant-region isotype as a distinguishing feature of T-cell subsets, the distinction must be sought elsewhere. One obvious question is whether the same gene pool encodes the  $\beta$  chain of all three major antigen-specific subsets. The original clones were isolated from helper cells. Saito *et al.*<sup>13</sup> have already shown that the same *C* segments are rearranged in cytotoxic cells, and similar analyses have now been extended to other cytotoxic cells and to suppressor cells both in man<sup>4</sup> and in mouse<sup>5</sup>. Hedrick *et al.*<sup>6</sup> have confirmed that the same *C* genes are both rearranged and transcribed in all of six cytotoxic cell lines analysed, and Toyonaga *et al.*<sup>4</sup> find rearrangements of the same *C* sequences in human helper and cytotoxic clones.

The position on suppressor cells is more complex. Toyonaga *et al.* report that their one suppressor clone shows rearrangement of the same *C<sub>β</sub>* sequence; but Hedrick *et al.* find rearrangement in only 2 of 14 suppressor clones: in the remaining 12, *C<sub>β</sub>* segments are deleted, along with very large regions of neighbouring DNA. Moreover, the two rearranged *C* segments lack *V* sequences. The weight of the evidence, then, is that the identified  $\beta$ -chain genes do not participate in antigen-specific suppression of the immune response. The major chromosomal deletions reported by Hedrick *et al.* are unlike anything seen in lymphocyte ontogeny before, and it is not clear what they mean. Saito *et al.*<sup>13</sup> have raised the possibility that if the same *C<sub>β</sub>* segments are expressed in helper and cytotoxic subsets, it remains possible that different *V<sub>β</sub>* pools may be active. This would be consistent with the different

## The Snark was a Boojum, probably

It would be disingenuous, in an article on the T-cell receptor, not to acknowledge the questions that have been widely raised about the true identity of the cDNA clone isolated in the laboratory of Susumu Tonegawa from a cytotoxic T-cell line and credited with encoding the  $\alpha$  chain of the receptor (Saito, H. *et al.* *Nature* 309, 757; and Winchester, G. *Nature* 309, 750; 1984).

Doubts were already being voiced at a conference on 'T-cell receptors, genetics, structure and function,' held at the Given Institute in Aspen, Colorado on 6–8 July, within a week of the appearance of the report in *Nature*, on the following grounds. Protein data on  $\alpha$  chains isolated from other kinds of T cells (helper cells) show that they are *N*-glycosylated (Kay, J. & Janeway, C.A. *J. Immun.*, in the press), whereas the sequence reported by Saito *et al.* in *Nature* has no *N*-glycosylation sites. Moreover, Tonegawa reported in Aspen that three ' $\alpha$ ' clones isolated from independent cytotoxic lines all contained the same *V* and *J* segments.

Leroy Hood (Caltech) argued in Aspen that such invariance was inconsistent with a role in antigen recognition, although others have pointed out that

there is a precedent in the  $\lambda$  light-chain genes of mouse immunoglobulin, which have only two variable-region segments. Moreover, variation in the exact site of the *V*-*J* join in the cDNA of the three T-cell lines analysed by Tonegawa does in fact generate some diversity that could, as he pointed out, have a significant effect on antigen binding, or on binding of the MHC molecules that form a necessary part of the antigenic stimulus of T cells. And as Tonegawa emphasized, the genomic DNA corresponding to the clone is rearranged and expressed exclusively in T cells and has clear homology to immunoglobulin, both in sequence and in organization, and thus fulfills all the important criteria for a T-cell antigen-receptor gene.

Thus at the time of the Aspen meeting it was unclear how far the discrepancies between the protein and the DNA data on the  $\alpha$  chain should be attributed to differences between man and mouse, or cytotoxic and helper cells; and how seriously the small size of the *V*-segment repertoire should be taken.

More definitive evidence is now becoming available and will this be the topic of a later article in *News and Views*.

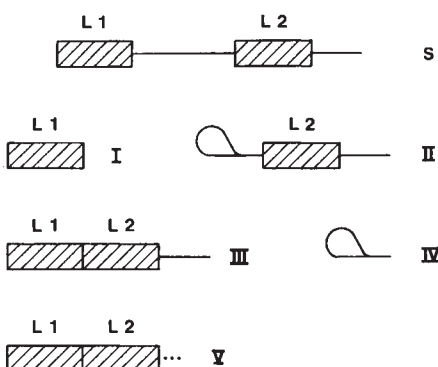
MHC-class responsiveness of these two subclasses, and Saito *et al.* point out that the homology between their cytotoxic-cell *V<sub>β</sub>* sequence and the helper *V<sub>β</sub>* sequence of Davis *et al.* is much lower than that between the *V* segments of immunoglobulin genes. It has since turned out, however, that very low homology is characteristic of *V* segments within the helper subset<sup>14</sup>. This discovery, its possible implications for antigen recognition by T cells and the elusive other half of the heterodimer will be the subjects of a second article in *News and Views*. □

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## Erratum

# Excisions of introns in lariat form



IN the article 'Excision of introns in lariat form' by Charles Weissman (*Nature* 311, 103; 1984), an incorrect version of Figure 1 was used. This was a result of a series of errors in the *Nature* office, for which we apologize. The correct figure is shown here on the left. It illustrates the structures of the products (I-V) of cell-free splicing of a substrate (S) RNA composed of two exons (L1 and L2) and two introns. Structure IV is the excised intron in lariat form.



## “Nuclear winter” to be taken seriously

IN two recent articles<sup>1,2</sup> John Maddox has criticized our technical analysis of the long-term worldwide consequences of nuclear war<sup>3</sup>. Our findings on what we have called nuclear winter evolved from, and were partly calibrated by, 12 years of related research on martian dust storms, the climatic consequences of volcanic explosions on Earth and the possible collision of an asteroid or cometary nucleus with the Earth at the time of the Cretaceous/Tertiary extinctions.

Given the importance and sensitivity of the subject, we took extraordinary measures to have our calculations reviewed by a large number of experts in atmospheric physics and chemistry, at a meeting specially convened for this purpose in April 1983, and by other means — well before the submission of the paper for publication. Our article refers to some 95 published scientific papers and reports in which further technical details can be found. We have discussed the technical issues with a large number of recognized experts in a variety of fields (which is not to say that all these experts endorse all of our conclusions). Most of the details of our mathematical models are already published, and a detailed 145-page description of our data sources and methodology has been widely distributed. A much more extensive discussion is in preparation.

Some apparently believe that unless nuclear winter is demonstrated beyond a shadow of a doubt, it is dangerous to discuss it in the scientific literature and in public forums. But this is not a subject amenable to experimental verification — at least not more than once. We hold, to the contrary, that open and informed debate on this issue is the only responsible approach, given the gravity of the potential climatic catastrophe we believe we have uncovered.

Whether Maddox has carefully read the work he is criticizing is open to some doubt. He seems to have missed the point that the major cause of nuclear winter is sooty smoke from fires rather than silicate dust raised from the surface. We clearly state that the greatest climatic effect is from smoke generated in the burning of cities. Nevertheless, in his first article and much of his second, Maddox surprisingly holds that the main climatic perturbation we derive is from dust. That this is not merely a careless use of words is made clear by his repeated comparison of our predictions of the effects of the nuclear war with the frosts produced by volcanic eruptions, exemplified by the work of LaMarche and Hirschboeck<sup>4</sup>, who find good agreement between the timing of some volcanic eruptions and temperature declines. Our previous work<sup>5,6</sup> shows that major volcanic explosions in the past few centuries may have produced optical depth perturbations of at most a few tenths, and

global temperature declines of at most one degree centigrade. These values are less than even the dust in a nuclear war would probably produce, to say nothing of the soot.

Maddox writes “The chief reason for this huge discrepancy between the consequences of a nuclear war and a volcanic eruption stems from the assumptions made about the quantities of dust carried into the stratosphere by the different events.” But our calculations unambiguously show that if there were no dust whatever injected into the atmosphere, and no aerosols of any composition injected into the stratosphere, severe climatic perturbations might nevertheless ensue from upper tropospheric soot. By his statements, Maddox also seems to be unaware that climatic effects of volcanic explosions are caused principally by sulphuric acid aerosols, not by silicate dust. Likewise, our results differ from those of the 1975 National Academy of Sciences report, largely because that study did not consider the injection of soot into the atmosphere from massive fires. But it is also true that the 1975 report did not carefully quantify the dust injection.

Maddox evidently believes that we have not justified or even stated the properties of the dust we employed in our calculation. In fact, we used direct measurements of the particle size distribution of dust produced by nuclear explosions, data on the properties of soils and observations of windblown dust. The log-normal/power law distribution function adopted, the complex refractive index of the dust, and other relevant parameters are explicitly stated.

Nuclear explosions are not volcanic explosions, and the differences between the two kinds of events far outweigh what they have in common. The relative importance of smoke in dominating the attenuation of sunlight in the immediate post-nuclear war environment was first noted by Crutzen and Birks<sup>7</sup>. Our calculations show that smoke is much more effective than dust per unit mass of aerosol, both for optical and for climatic perturbations, because of the greater absorptivity and finer particle size of the former.

Maddox criticizes Covey *et al.*<sup>8</sup> for applying their “new model” to nuclear winter before its applicability has been demonstrated in more conventional calculations of the general circulation. The ‘new model’ is in fact the Community Climate Model of the National Center for Atmospheric Research, which has been in general use for some years. In our *Science* paper<sup>3</sup> we explicitly noted that the great heat capacity of the oceans will have an ameliorating effect on the post-nuclear war temperature perturbations even in continental interiors, and made a rough estimate of the magnitude of this amelioration. As Covey *et al.* make clear, their

three-dimensional general circulation model, as well as one, two and three-dimensional models at Lawrence Livermore National Laboratory and at the Computing Center of the USSR Academy of Sciences, are all in good agreement, in hemispheric average, with our published results. Moreover, Covey *et al.* show that the effects could be significantly worse than we had stated, in that “quick freezes” could occur in a matter of days in areas far distant from the combatant nations, as well as showing that calculated perturbations to the global circulation could bring dust and soot rapidly to the tropics and into the Southern Hemisphere, as we had originally proposed.

There are many points about nuclear winter that require further work, both theoretical and experimental. We have never suggested that the last word has been said on this subject<sup>9</sup>. We have, however, run some 50 different cases, to study uncertainties and sensitivities, in many of which, including those we consider most likely, the climatic effects are very severe.

There are contingencies, which we consider unlikely, that could mitigate the severity — for example, if the smoke is everywhere efficiently scavenged from fire plumes, or if there is persistent mesoscale patchiness in the aerosol cover. But there are also circumstances, some of them very likely, in which the effects could be considerably worse. For example, the smoke from fires set by one nuclear explosion over a city can be propelled rapidly by a later explosion to very high altitudes, where it is immune to scavenging mechanisms. By creating channels for bombblight and by drying, a first nuclear explosion over forests can enhance the burning produced by a subsequent nuclear explosion. We have also entirely ignored the effects of tactical nuclear weapons; there are now almost 35,000 of them in the world inventories, many with yields greater than those of the bombs that destroyed Hiroshima and Nagasaki.

The dramatic restructuring of the Earth's atmosphere by injected aerosols moves the lower atmosphere towards isothermality and the upper atmosphere towards a major thermal inversion, as shown in our *Science* paper. In a fully interactive calculation, this restructuring would significantly prolong the duration of the climatic effects following a nuclear war. The snow/albedo and sea ice/thermal inertia (see ref. 10) feedback effects also act to extend the duration of nuclear winter.

As one measure of the importance of the nuclear winter problem, within the past few months major research efforts, costing at many millions of dollars, have been authorized in the government laboratories of both the United States and the Soviet Union, and still larger programmes are under urgent consideration. Spokesmen for the US Department of Defense have recently testified that they take nuclear winter very seriously<sup>11</sup>.

In the closing paragraph of our *Science* paper and elsewhere in the text and notes, we summarize our view of the limitations of this research and express the hope that, because of these new dangers of nuclear war, "the scientific issues raised (here) will be vigorously and critically examined". Many members of the scientific community, ourselves included, are now engaged in such an examination.

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JOHN MADDOX REPLIES — My objective, in the first brief reference to this work and in the second reference, was to remind readers, either of the original works or of *Nature*, that these calculations are uncertain. Plainly I should have made more of the smoke and less of the dust, but the conclusion remains — that the assumptions on which these calculations are based, and the computational techniques themselves, are respectively (but necessarily) so uncertain and imprecise that it would be folly to regard them as more than suggestive, but interesting and even valuable (as I said) on that account.

Uncertainty about the scale on which nuclear war might be waged is probably unavoidable, and I have no complaint at the scenario used; it serves the purpose. The uncertainty about the quantities of smoke generated, the height to which smoke would be carried, the local meteorological effects with which its generation would be attended and the rate at which particles would aggregate and be washed out cannot be processes that could affect the quantity of smoke available for redistribution over the surface of the Earth by one or more orders of magnitude.

Even the climatic modelling is susceptible to sources of error not yet taken account of. The essence of the nuclear winter is a global temperature inversion: how stable is this against lateral variations? The point has not to my knowledge been tested. And there has been no investigation of the likelihood that such an inversion

would be formed, by the diffusion of carbon particles over the surface of the Earth, without provoking the vertical movements in the atmosphere whose effect might well be to inhibit the temperature inversion altogether. I acknowledge, of course, that this is a difficult and, for the time being, an intractable problem; the authors have followed other modellers in solving the easy problems first. On such a matter, certain to stir the public imagination, it seems to me improper that the results of calculations should be published even in sober language without a warning to all potential readers of the pitfalls there must be. This is doubly unfortunate when, as on this occasion, a purportedly scientific publication is so fully amplified by popular articles, first in *Parade*, most recently in *Scientific American*.

I do not believe that the authors are politically motivated, except in the most general sense that they believe they may help to save the world. But I think it disingenuous of them to overlook the ways in which their conclusions may be used by politicians in other causes, often of a political character. □

## Amino acid transport systems

SIR — We are concerned about the problem of making simple and unambiguous reference to well-characterized amino acid transport systems, and we indicate here how we aim to minimize unnecessary further complexities in our use of abbreviations for such systems.

Where a new transport system appears to be simply a variant of a known system, we plan to recognize this close relation with a terminology such as A1, A2, ..., reserving previously unused Roman letters only for more distinctly different systems. In this way we hope to avoid exaggerating complexity. We hope also to avoid evoking any single amino acid where it may be misleading because the system later turns out not to be approximately specific for that amino acid.

For systems for basic amino acids in general, where the mediator appears to accept these amino acids only in a cationic form, we plan to use the symbol  $y^+$ , the positive charge indicating the apparent selectivity for the cationic form. (This symbol omits an initial letter L earlier included in  $Ly^+$  (ref. 1), whereby a single amino acid was too strongly evoked.) Conversely, for systems for dicarboxylic amino acids where these appear to be accepted only in their anionic form, we plan to use the symbol  $x^-$ . The plus and minus signs do not merely emphasize the requirement that the amino acid substrates have a positively or a negatively charged side chain; they also allow for the possibility that systems will later be found that specifically recognize and require the uncharged side chain group  $-COOH$  or  $-NH_2$ . We plan then to add a

single subscript letter such as A, G, or C (for example,  $x_A^-$ ) where it seems desirable to indicate that one of these anionic systems is rather specific for aspartate or glutamate, or includes cystine among its substrates. An important  $Na^+$ -independent system for anionic amino acids,  $x_C^-$ , does indeed include cystine and glutamate among its substrates. When a transport system  $x^-$  and  $y^+$  is  $Na^+$ -dependent, we propose to indicate it by making x and y capital letters,  $X^-$  and  $Y^+$ . Specification as to the cell type or organelle under consideration will generally be needed when referring to a particular system, especially for a system apparently peculiar to one or a few tissues.

The terminology x, y may be seen as part of a scheme x, y, z, in which z stands for 'zwitterionic'. This leaves the as yet unassigned letter z for one or all systems for zwitterionic amino acids. The letter N is preempted for another meaning<sup>2</sup>.

We do not necessarily mean to complicate matters by these ideas, by changing nonconforming abbreviations which may be regarded as already well established, even the capital L in the classical system<sup>3</sup>, although a lower case l (as in l3, l4, ...) will seem appropriate for new similar  $Na^+$ -independent systems. We think it is important that a transport system should be characterized in detail before a code is designated for it. The various designations need even then to be regarded as provisional, as the homogeneity of a given component of transport is itself a provisional conclusion, and two components may turn out to be less similar than was at first supposed.

We invite colleagues who come to participate in identifying such transport systems to join us in a general effort for reasonable simplicity.

1. Christensen, H.N. & Liang, M. *J. biol. Chem.* **241**, 5542-5551 (1966).
2. Kilberg, M.S., Handlogten, M.I. & Christensen, H.N. *J. biol. Chem.* **255**, 4011-4019 (1980).
3. Oxender, D.L. & Christensen, H.N. *J. biol. Chem.* **238**, 3686-3699 (1983).

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# Where is the revolution?

*Electronic publishing will bring a new vitality to science. Michael Buckingham evaluates the promise and the problems.*

WHEN Gutenberg invented moveable type, there was no doubt about how the new technology would be applied. Electronic publishing is in no such happy situation. The technology exists and certain benefits have long been clear, not least scientifically. But where, the scientist may reasonably ask, is the revolution in communication which will change his working life?

Electronic information systems such as *Chemical Abstracts*, *Index Medicus* and *Excerpta Medica* have been with us for some years, and are recognized to offer a better service than their printed equivalents. Hitherto, these services have entailed asking library staffs to conduct a search, but increasingly desk-top computers and word processors are being used to gain entry to them, while managers of the database systems are offering means of access which are simpler and cheaper than before. These developments give scientists freedom and flexibility, as well as the obvious physical convenience of being able to search electronically throughout many years' issues rather than physically to manhandle the printed volumes, while the

**... even enthusiastic supporters of electronic publication have to admit that presentation suffers in the process.**

quality of the searches is improved by using combinations of search terms in a way that would be impracticable with printed text. For these secondary services, electronic access continues to gain ground at the expense of the printed version.

But what of the primary literature? For several publishers, the first step has been simply to make the full texts of some of their most important primary journals into computer databases. Thus the American Chemical Society last year made a series of its titles available in this way while, in the biomedical field, Elsevier has offered the rapid-publication journal *IRCS Medical Science* as a full-text database updated simultaneously with the appearance of the printed issues. This year, *The Lancet* and the *British Medical Journal* will become available in database form. Books are not being neglected. Saunders and Churchill Livingstone are active in making their medical textbooks available electronically; the Martindale *Extra Pharmacopeia* is now on-line; and Wiley has made its *Medical Research Directory* into an on-line file which reports work in progress in academic, hospital and government institutions.

The benefits for the reader of electronic access can be substantial. Because of postal

delays alone, the advantage of speed is considerable — the reader of a British journal in the United States can see articles up to a month ahead of delivery of the printed issues and can also circumvent the delays of circulation lists. Moreover computers can be used to search past issues and deliver the text of articles immediately, without activating the ponderous processes of inter-library loan. The user of a textbook has the benefit of always reading the latest edition. But even enthusiastic supporters of electronic publication have to admit that presentation suffers in the process. Reading articles on a terminal screen is out of the question. Computer printout, even on the best office printers, lacks the clear, compact presentation of good typography, while the loss of illustrations may be unacceptable. Where papers are short, and rapid publication is a prime objective, then the advantages of electronic publication can be decisive. A 3,000 word paper, on the other hand, is all but totally unmanageable on-line, at least with present technology.

These developments are obvious first steps, but we are still far short of a revolution in scientific communication. Making the text of more journals available electronically is not the answer, nor is the simple speeding up of the information process by making titles, abstracts and bibliographic data accessible through databases. The real task is to develop electronic forms of communication based on a pragmatic understanding of the medium and of the needs of scientists. What can electronic publishing offer that printed journals cannot match? And how can such services better the convenience of dropping into a library at regular intervals to scan favourite titles and look through *Current Contents*?

The early emphasis on converting printed databases to electronic form has

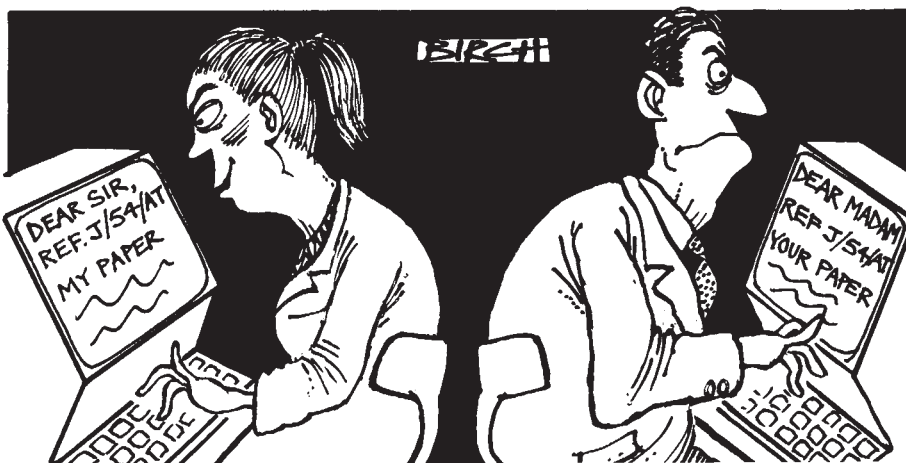
tended to overshadow one of the main advantages of computers — the fact that they offer an *interactive* means of communication. Once entered into a system, new information can be collated with existing entries, immediately updating the response to new enquiries. Thus we have an opportunity to introduce a new dynamism into scientific communication, countering the main weakness of the traditional journal.

I have elsewhere said that reading traditional journals is like entering a well kept cemetery; the papers stand in ordered

**... reading traditional journals is like entering a well-kept cemetery.**

rows, timeless and unchanging. Journals emerged as means of scientific communication when public lecture and debate became impracticable, but much was lost in the process. Scientists continue to talk to each other, of course, at congresses and symposia, but these can be attended only by a small proportion of the workers in any field. And the bibliographic problems associated with recording the proceedings of many meetings mean that much information is effectively lost once the meeting is over.

The concept of an electronic journal receiving manuscripts by telephone and combining a comment and message service is not, of itself, new. Much of the early work undoubtedly became entrapped in the technology — summed up in the immortal words of John Senders: "I have seen the future and it doesn't work" — but things are changing. In Britain, the BLEND experiment (Birmingham and Loughborough Electronic Network Development), sponsored by the British Library, has provided a successful form of electronic communication for participants, though it has not yet been a vehicle for a great deal of formal original publication. And there are some commercial developments, notably in medicine. In Britain, Elsevier is launching *Clinical Notes On-Line*. This will be an electronic information service, based on submitted and reviewed material, in which



feedback from readers will play a crucial role. In the United States, 3M Inc. is sponsoring the development of a "Bulletin Board" service for the medical profession; the Minnesota Medical Conference Tree has 21 sections ranging from cardiology to general surgery to which physicians can contribute ideas.

These services have emerged to serve particular specialist groups. To meet the

**... if publishers have to learn new skills, it is reasonable that editors and contributors should do so as well.**

needs of the vast range of basic scientific research will be more demanding. In particular, it will be necessary to define the formal status of electronically published work — the role of the peer-review system has to be protected for exactly the same reasons as apply to the printed word. The basis for citation of the work must be equally clear, and some form of archival record must exist. These considerations probably mean that there will still be a need for a printed form of each record, although this need not necessarily be bound and circulated as a conventional journal.

Because of the continuing role of printed publications it is entirely possible that electronic debating chambers will grow piecemeal from the electronic publication of existing journals. *IRCS Medical Science* now offers a comment facility, and *The Lancet*, with its strong Letters tradition, is almost an instant electronic forum. But where a journal is not already published electronically, it is arguably easier to start afresh. Whatever the case, contributions will certainly have to be vetted before being made available; editors will still have to edit, and not be seduced by the ease of transmitting the raw material submitted to them; and authors will have to be discouraged from allowing the opportunity to add postscripts to become a substitute for forethought. But if publishers have to adapt and learn new skills, then it is reasonable to ask that editors and contributors should do so as well.

There is a further factor, which being financial may well be decisive. All electronic services charge the reader directly according to the amount of information received. As things are now, a library is a general overhead provided at little direct cost to the individuals using it. Adjusting to a pay-as-you-go system will be traumatic; budgetary responsibility will move away from the librarian to the reader. The true challenge for all concerned, but most of all for the publishers, is to produce services that not only realize the tremendous potential of electronic publication but give immediate value for money. That challenge is a familiar one in selling newspapers but augurs a revolution for scientists and their publishers. □

Michael Buckingham is Managing Director of Elsevier-IRCS Ltd, Lancaster, UK.

## New journals June 1982 to May 1983

CRITERIA for journals to be considered for review in this issue were circulated to publishers earlier this year, and were also published in *Nature*. They were that:

- (i) the first number appeared, or the journal was re-titled, between June 1982 and May 1983 (although journals not covered in the previous review issues were also considered);\*
- (ii) the journal appears at least three times a year (two exceptions have been made to this rule);
- (iii) the main language used is English;
- (iv) where possible four issues should be made available for review, the first, the most recent, plus two others.

Last year's journals review supplement covered publications appearing between June 1981 and May 1982 and the second cut-off date, May 1983, allows for enough issues of a journal to have been published for a reasonable sample to be available for judgement (most are quarterlies). A spread of four different issues is taken as being the usual minimum on which reviewers' comments can be based.

\*See *Nature* 293, 341-369 (1981); 299, 491-514 (1982); and 305, 477-497 (1983).

Several journals known to satisfy the above criteria were not submitted for review, or arrived only in August or September, and therefore are not covered in this issue. It proved impossible to find reviewers for other, doubtless worthy journals, while some titles were considered to be of marginal interest to *Nature's* audience.

The brief given to reviewers was to limit themselves to comment on the publications sent to them, and to avoid discussion of general questions of periodical publishing. Opinions expressed in the reviews are based on a sample, and apply to mid-1983 at the latest. As in previous years, the preponderance of journals in the biological sciences reflects the bias of the material submitted for review, and the shortest possible (unconventional) abbreviations of titles have been used in the reviews.

Details of editors and frequency of publication, and the basic annual subscription rates appearing at the top of each review are given in most instances for 1985. These details are not complete in all cases, and readers interested in subscribing to a particular journal should check the rates with the publisher concerned.

## Index to reviews

|                                                     |     |                                                                                      |     |
|-----------------------------------------------------|-----|--------------------------------------------------------------------------------------|-----|
| Annales Geophysicae                                 | 326 | Journal of African Earth Sciences                                                    | 327 |
| ATLA (Alternatives to Laboratory Animals)           | 321 | Journal of Biological Response Modifiers                                             | 318 |
| Behavioral Neuroscience                             | 323 | Journal of Carbohydrate Chemistry                                                    | 316 |
| Biological Agriculture & Horticulture               | 317 | Journal of Cereal Science                                                            | 316 |
| Bio/Technology                                      | 317 | Journal of Comparative Psychology                                                    | 323 |
| CAL: Computer Applications in the Laboratory        | 313 | Journal of Metamorphic Geology                                                       | 326 |
| Cancer Investigation                                | 318 | Journal of Microbiological Methods                                                   | 320 |
| Cell Biochemistry and Function                      | 320 | Journal of Molecular Graphics                                                        | 314 |
| Chinese Journal of Oceanology and Limnology         | 325 | Journal of Sports Sciences                                                           | 321 |
| Clinical & Experimental Metastasis                  | 318 | Laser and Particle Beams                                                             | 328 |
| Clinical Physiology and Biochemistry                | 321 | Lens Research                                                                        | 322 |
| Computer Enhanced Spectroscopy                      | 315 | Materials Chemistry and Physics                                                      | 329 |
| Computer Graphics Forum                             | 312 | Mechanics of Materials                                                               | 329 |
| Computers and Standards                             | 311 | Molecular Biology & Medicine                                                         | 318 |
| Continental Shelf Research                          | 325 | Neurochemical Pathology                                                              | 323 |
| Crop Protection                                     | 317 | New Generation Computing                                                             | 311 |
| Crop Research                                       | 317 | New Ideas in Psychology                                                              | 323 |
| Diagnostic Immunology                               | 319 | Nucleosides and Nucleotides                                                          | 316 |
| Diagnostic Microbiology and Infectious Disease      | 320 | Nutrition and Behavior                                                               | 322 |
| Earthquake Prediction Research                      | 328 | Plasma Chemistry and Plasma Processing                                               | 329 |
| Experiments in Fluids                               | 330 | Quantitative Structure-Activity Relationships in Pharmacology, Chemistry and Biology | 314 |
| First Break                                         | 326 | Reactive Polymers, Ion Exchangers, Sorbents                                          | 315 |
| Geochemistry                                        | 326 | Renewable Sources of Energy                                                          | 324 |
| Image and Vision Computing                          | 313 | Risk Analysis                                                                        | 324 |
| Integration: The VLSI Journal                       | 311 | Solvent Extraction and Ion Exchange                                                  | 315 |
| Interfaces in Computing                             | 311 | Sports Medicine                                                                      | 321 |
| International Journal of Developmental Neuroscience | 324 | Technology and Science of Informatics                                                | 311 |
| Isotope Geoscience                                  | 327 | Trends in Biotechnology                                                              | 317 |



# Computing communications

W.F. Clocksin

## New Generation Computing: An International Journal on Fifth Generation Computers.

Editor-in-chief T. Moto-oka.

Springer-Verlag. 4/yr. DM 250, \$96.

**Integration: The VLSI Journal.** Editor-in-chief L. Spaanenburg.

North-Holland. 4/yr. Dfl. 250.

**Technology and Science of Informatics.** Editor-in-chief Bertrand Meyer.

North Oxford Academic, 242 Banbury Road, Oxford OX2 7DR, UK. 6/yr. £60, \$96.

THE conventional computer designs established by Alan Turing and John von Neumann have begun to encounter numerous difficulties; most particularly, low software productivity and insufficient processing power on non-numerical data have led to the so-called "software crisis". In response, promising new ways of thinking about computation have emerged at the intersection of hardware and software studies. One result has been VLSI (Very Large Scale Integration), a semiconductor technology which makes possible the fabrication of electronic circuits having hundreds of thousands of transistors on an area the size of a fingernail. On the software side, it is becoming increasingly appreciated that the mathematical properties of functional and relational programming languages (such as Prolog) are suitable for specifying logical inferences and computations in an inherently parallel fashion. Such parallelism promises to make effective use of the host of computing elements provided by VLSI technology.

These interconnected technical issues, taken together with planned applications in natural language processing and knowledge engineering, constitute the new generation of computing, and a journal sympathetic to these aims seems sure to be well received. *New Generation Computing* (NGC) shows every sign of fulfilling its potential as the forum for researchers in this area. The need for a new journal is well justified, not the least because of the appalling publication delay in most computer science journals. Credit is due to the Institute for New Generation Computing in Japan for instigating this journal, and for showing from the start a concern for technical and scientific issues.

All of the papers appearing in NGC are in English, but while most of them have thus far originated from Japanese researchers I predict that representation will become more widespread as the field matures. The publisher has done a fine job of packaging and presentation, although in some articles the standard of English (an unfamiliar language to many contributors) leaves much to be desired, requiring considerable imagination on the part of the reader — greater editorial intervention

seems to be called for in this regard. Similarly the scientific standard of the papers is somewhat patchy, although this will undoubtedly improve in the future. Each issue of the journal contains about eight papers, many of them short notes of some six pages in length, and there is usually an editorial and a non-technical "leading article" of a more general nature. The journal needs to do some growing up, but my overall impression is that NGC will prove to be indispensable to those who need to be kept informed of developments as the fifth generation approaches.

*Integration: The VLSI Journal* is a natural partner to NGC. The title *Integration* is an appropriate if unintended *double entendre*, for the coverage of this journal is of interest to the mathematician and computer scientist as well as the electrical engineer. Editorial policy stresses the mutual benefit of communication between these practitioners, and this is a very healthy sentiment which I hope propagates more widely to industry. Certainly, *Integration* will be a valuable addition to the literature serving the computer scientist and engineer.

The papers published in the journal rise to the challenge laid down by the editors and are of high standard. Like NGC, *Integration* has an admirably short publication delay, and also includes a number of departments — letters to the editor, news and a calendar of events, and abstracts of a dozen or so relevant papers published elsewhere. Quality of illustrations is important in reports of VLSI work, and monochrome photographs and half-tones are reproduced to a high standard. As yet, no colour plates are in evidence, however, and no advice on submitting coloured photographs is given, but colour does not seem to be out of the question because both the cover and one advertisement sport the appropriate hues.

Compared with *Integration*, *Technology and Science of Informatics* (TSI) has a wide coverage and is intended to appeal to the more general audience of computer professionals. The journal is a cover-to-cover translation of the French publication, *Technique et Science Informatiques*, and classifies its papers under the three headings "Surveys",

"Applications" and "Research". Each paper is introduced by a few paragraphs of commentary by a member of the editorial board. In addition, there are plenty of photographs and line drawings, and there is a relatively large departmental section with correspondence, reviews, bulletins and calendar. The four or five papers appearing in each issue are not dated, giving no obvious clue to publication delay.

It would be natural to find a French emphasis here and, indeed, most of the

## TECHNOLOGY AND SCIENCE OF INFORMATICS

reviews are of books written in French and the format is identical to that of the parent journal. The publishers have clearly gone to some trouble to translate the papers well, though there is the occasional bloomer; in one article on Unix, for example, the phrase "branching catalogue" should have been rendered "tree-structured directory".

The advantage of TSI is that it offers a direct line to the French computing community, and it will meet the needs of those especially interested in knowing what is happening across the Channel. □

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## Standard reading

A.M. Addyman

### Computers and Standards.

Editor-in-chief John L. Berg.

North-Holland. 4/yr. Dfl. 262.

### Interfaces in Computing.

Editors R.W. Dobinson and P.N. Clout.

Elsevier Sequoia. 4/yr. SwFr. 195, \$92.75.

THE advent of *Computers and Standards* (CS) reflects a growing awareness of the need for standards within the computing industry. Unlike most journals in the general area of computing, CS is not limited to one topic such as programming or computer architecture. Rather, it addresses standardization, which affects virtually all aspects of computing. Indeed there is an increasing number of branches of the subject in which the only effective way forward is through the standards process.

Most of the information in CS appears in the form of papers and commentaries; why some contributions are classified as papers and others as commentaries is not clear — to me at least. The quality of the material is, generally, very high, which is only to be expected as many of the authors are well-known and very active in standardization work. Recent issues have contained contributions on the programming language

## A choice of new journals from Karger

### Acute Care

International Review of Critical Care Medicine

An instructive guidance for all specialists working in intensive care units who require access to the newest developments in emergency care procedures.

Editors: M.H. Weil, North Chicago/Ill.

E.C. Rackow, Chicago/Ill.

1985: Volume 11 with 4 issues

Institutional subscription: SFr. 218.-

Personal subscription: SFr. 109.-

### Digestive Surgery

A new reference to basic research as well as advances and technical innovations in a diversity of fields relating to surgery of the alimentary tract.

Editors: E.H. Farthmann, Freiburg i.Br.

L.F. Hollender, Strasbourg

1985: Volume 2 with 4 issues

Institutional subscription: SFr. 246.-

Personal subscription: SFr. 123.-

### Experimental and Clinical Immunogenetics

A guide to the explosive growth of research in immunogenetics and its emerging clinical applications.

Editor: K. Bauer, Heidelberg

1985: Volume 2 with 4 issues

Institutional subscription: SFr. 198.-

Personal subscription: SFr. 138.60

### Pediatrician

International Journal of Child and Adolescent Health

Problem-orientated topic issues pertinent to practitioners for the maintenance of international expertise on psychological problems and medical disorders with special emphasis on ambulatory pediatrics.

Editor: D.W. Kaplan, Denver/Colo.

1983/85: Volume 12 with 4 issues

Institutional subscription: SFr. 180.-

Personal subscription: SFr. 90.-

### Pediatric Neuroscience

Successor to 'Child's Brain'

Experimental and clinical studies featuring new information and observations in pediatric neurosurgery as well as neurology, neuroradiology and neuropathology.

Editors: E.B. Hendrick, Toronto/Ont.

D.H. Reigel, Pittsburgh/Pa.

1985: Volume 12 with 6 issues

Institutional subscription: SFr. 330.-

Personal subscription: SFr. 181.50

### Psychopathology

International Journal of Descriptive Psychopathology, Phenomenology and Clinical Diagnostics

Research centered on the concepts, models, and diagnostic categories that guide the practice of psychiatry and help illustrate the dynamics of psychopathologic phenomena.

Editors: P. Berner, Wien, E. Gabriel, Wien

1985: Volume 18 with 6 issues

Institutional subscription: SFr. 254.-

Personal subscription: SFr. 177.80

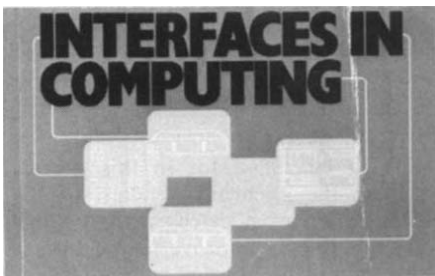
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COBOL, open systems interconnection (OSI) and database management systems, in addition to a number of articles dealing with the process by which standards are produced and the implications of standards production. As well as the main contributions, each issue of the journal contains details of standards activities and standards publications.

Much of the content of CS will be of particular value to organizations in the com-



puting industry, because the development of standards affects the market for their products. The libraries of educational establishments will find there is less demand for the journal, unless their researchers are active in such fields as programming languages, graphics, databases or computer networks. The key to the success of CS is the fact that the information it contains is generally very difficult to obtain unless one is actively involved with the work. However, the publishers will need to ensure that the delays between the receipt and publication of papers are reduced, because of the perishable nature of much of the information on standards.

Elsevier Sequoia, the publishers of *Interfaces in Computing* (IC), cannot be criticized for tardy publication — the time from receipt of a manuscript to its appearance in print is typically six months. This is essentially a practical journal — like *Software: Practice and Experience*, for example — and it has a fresh, down-to-earth quality. Many of the papers have been written by authors who have created something and are writing from experience; the paper is the result of their work not the reason for it!

Even though there are interfaces in every piece of computer hardware and software, the variety of topics embraced by IC is truly surprising. Subjects covered recently include computer transcription of machine shorthand; device-independent graphics; a pipelined multimicroprocessor system; Cambridge Ring interfaces; CAMAC; and entity relational database systems.

The scope and nature of this journal is such that many computing professionals will find at least one paper of interest in each issue, though the large number of contributions dealing with hardware will probably mean that computer engineers will find more than their software counterparts. Certainly, I will add it to my list of journals to be read. □

A.M. Addyman is in the Department of Computer Science at the University of Manchester.

## Graphic applications for Europe

Julian Gallop

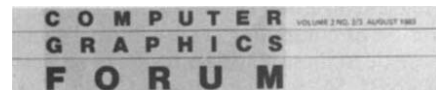
### Computer Graphics Forum.

Editors Günter Enderle and David Duce.  
North-Holland. 4/yr. Dfl. 222.

COMPUTER graphics has become accessible to the layman since personal computers with graphics have become more sophisticated and cheaper, and many magazines are available to such readers. The professional user, practitioner or researcher has different requirements, however, and there are far fewer relevant journals available.

*Computer Graphics Forum* (CGF) is the official publication of the European Association for Computer Graphics (Eurographics) and is now in its third volume. (For an individual, joining Eurographics and thereby obtaining CGF free is cheaper than buying the journal separately.) The text is edited and typeset with computer assistance and the quality of production is good and (after the first volume) uniform.

A typical issue contains refereed technical articles; reports and announcements; and Eurographics association news. The technical articles (generally 5–15 pages in length) cover various applications of computer graphics (for example, in chemistry, molecular biology and population planning) and studies in theory or techniques (for example, contouring, picture processing and geometric modelling). The number of articles varies perhaps too widely between one issue and the next; the average is about four to five an issue. This is a rapidly changing field and CGF aims to publish refereed articles



quickly. In general, this is achieved, but there has clearly been some publication delay when colour photographs are reproduced on the same pages as text. Occasionally, colour photographs seem to have been included gratuitously and do not add information, notably the pictures of a VDU screen that is virtually a single colour.

Part of CGF is devoted to reports, announcements and surveys. Important events in Europe, and some outside, are announced and reported, including meetings, workshops and conferences. In this part of the journal a book review section has recently been started.

There are parallels between CGF and *Computer Graphics*, published by the Siggraph Association (Special Interest Group on Computer Graphics). However, while international, the latter has a strong North American emphasis and CGF is



providing a focal point for refereed technical articles of European origin. In addition, the Siggraph technical contributions are largely concentrated on its conference proceedings and on the very valuable and thorough annual bibliography on computer graphics.

By contrast with other journals in this area, a strong theme in CGF is the standardization of computer graphics. Progress reports, articles on the interpretation and use of standards, and a survey of implementations of the computer graphics programming Standard have been published. Also, an algorithms section has been announced and if successful will encourage the exchange of software using the Standard. □

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## CAL tech

D.J. Malcolm-Lawes

### CAL (Computer Applications in the Laboratory).

Editors Lawrence P. Forsley, David W. Grant, Rudolf E. Kaiser and Anthony J. Rackstraw.

*Alfred Heuthig Verlag, PO Box 102869, D-6900 Heidelberg 1, West Germany. \* 6/yr. £30, \$50.*

AMIDST the welter of magazines feeding the public appetite for computing at home, we are now seeing the appearance of publications which attempt to provide a forum for the exchange of information on the scientific uses of microcomputers. One such journal, *Laboratory Microcomputer*, was reviewed in *Nature* last year (305, 495; 1983). Another is *Computer Applications in the Laboratory*, launched in March 1983, which apparently wishes to be known as CAL.

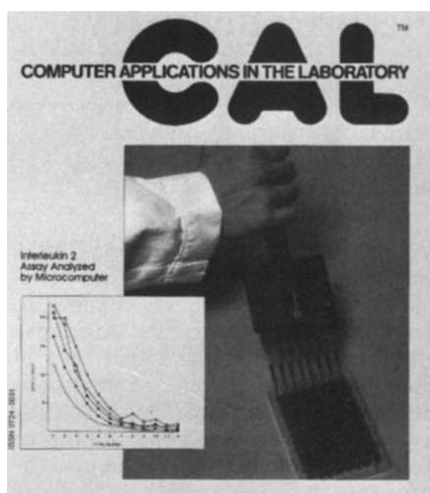
As an avid reader of popular computer magazines and a user of laboratory microcomputers, I was moderately excited at my first contact with CAL, and felt that here perhaps was a journal designed for people like me. Sadly this excitement has faded as more issues have appeared.

The publication is well turned out and has many of the features of a popular computer magazine: a glossy colour cover, plenty of photographs, large advertisements, and an editorial and a pattern of sections in each issue. Essentially the magazine is divided into four — news, features, series and “scientific originals”. The news items are most commonly one-page articles/advertisements reporting a new computer or program or a conference — FORTH advocates and Perkin-Elmer

have dominated so far. Features vary from short reviews of books and programs (e.g. BASIC compilers for Applesoft BASIC), to literature searching, to word processing (although both of the word-processing articles have been on the Apple Writer). The series in the first year have included “Personal computers in the laboratory”, “Interfacing”, “Learning FORTH” and “6502 made easy”. In each case division of the text into different issues was unfortunate because the individual episodes so far have occupied only a small amount of space, and in some instances have contained very little worthwhile information.

The criticisms to be levelled at these three sections are that the hard information content is too low and that selection of topics is too restricted (applications other than chromatography and computers other than the Apple would be of interest). Further, the excessive use of half-page cartoons conveys the impression of a vain attempt to hide a lack of editorial material.

The original work (including short communications) reported in the final section comes from a wider range of contributors and is consequently much more varied. Articles have covered applications of several popular microcomputers, and even the DEC Minc mini. Most of them so far have been on software, covering topics such as the resolution of HPLC peaks, measurement of lung function in babies and children, and electrothermal atomic absorption spectroscopic analysis. The last issue available for review (1/84; volume numbers are not used) contained one scientific original (4 pages) and one short com-



munication (3 pages). That is seven pages out of a total of 60. Not all issues are that low on papers, but even so the serious content is disappointingly small.

There is indeed a place for a publication such as this, half journal and half magazine. But if CAL is to satisfy its readers, and not just its advertisers, the editors will have to include more meat and less froth. □

*D.J. Malcolm-Lawes is Royal Society Research Fellow in the Physical Sciences at King's College, University of London.*

## Need for greater vision?

P.W. Hawkes

### Image and Vision Computing.

General editor Keith Baker.

*Butterworth. 4/yr.*

*£82, \$164.*

BRANCHES of science are commonly referred to as fields but the application of computing to imaging and vision is more like a wide sea, spreading over robotics, medicine, military reconnaissance, astronomy, microscopy and a host of other subjects. Thus far it is a shallow sea, however, for despite all the theoretical work there are far more people paddling than swimming. Inevitably, progress is being made on related practical problems in different domains of application. It is in the hope of countering the duplication of results in specialist serials, by collecting together papers of potential interest in several disciplines, that *Image and Vision Computing* (IVC) has been launched by Butterworth.

So far, its success is limited. There are indeed practical papers of general interest but there are others that would have been more widely read in a specialist journal. In particular there are numerous theoretical contributions for which natural homes already exist — the *IEEE Transactions on Pattern Analysis and Machine Intelligence*, *Computer Vision, Graphics and Image Processing* and *Pattern Recognition* spring to mind. Furthermore, there are only five or six articles per issue, so few that workers in this field will have to look at the other, more-specialized publications anyway. So the case for this new interdisciplinary journal is not very strong.

In addition to original articles, each issue includes a list of related publications in other journals; this list is very short (a dozen or so per issue) but includes long abstracts. It is followed by one or two book reviews (the May 1984 issue contains a review of a book published in 1982), a news feature entitled “Insight”, a page or two of “Product News” and a calendar of forthcoming meetings. The latter is useful and easy to consult, though it is not obvious what Applications for Management and Productivity will be discussed at CAMP '84 in Berlin!

It seems to me that there is a serious danger that IVC will attract material difficult to place elsewhere. These first issues do contain some extremely interesting articles—but there will have to be far more of them if the journal is to occupy the place to which its editor aspires. □

*P.W. Hawkes is Maître de Recherches at the Laboratoire d'Optique Electronique du CNRS, Toulouse.*

\*Also 97 Elgin Crescent, London W11 2JF, and 611 Broadway, New York, NY 10012.

## Drug design centred

J.M. Midgley

### Quantitative Structure-Activity Relationships in Pharmacology, Chemistry and Biology.

Editors Joachim K. Seydel and F. Darvas.

Verlag Chemie. 4/yr. DM 280, \$168.

THE elaboration of molecules which will selectively control biochemical processes in a predictable manner is one of the ultimate goals of drug research. Drug design has been defined as the application of rational methodologies which result in a new drug (either through total innovation or via the optimization of a lead compound), and in the past two decades there has been a rapid development in techniques for elucidating quantitative structure-activity relationships (QSAR) for many compounds possessing diverse biological activities.

An indication of the activity in this field is the emergence of a specialist journal, *QSAR in Pharmacology, Chemistry and Biology*, which has a distinguished editorial staff drawn largely from academia in Europe and the United States. The purpose of the journal is "to collect contributions on all aspects of the subject embodied in its title", in particular theoretical and methodological aspects and innovative applications of QSAR. Each of

the four issues published annually contains about four refereed contributions (averaging five to six pages in length) of original research, and a balance of subject matter within the editorial remit has been achieved so far. The articles are clear and succinct, and their scientific standard is comparable to those of a similar nature appearing in established journals. The papers largely originate from Europe and the United States, and the significant industrial contribution reflects the growing economic (as well as scientific) importance of QSAR.

Somewhat surprisingly, there is no editorial comment or opportunity for correspondence and the remainder of each issue is taken up with approximately 60 abstracts of publications related to QSAR; these are comprehensive and drawn from diverse sources, but may be too dated to excite the *aficionado*. The whole is produced in a modern two-column format, with clear print and illustrations.

At the price the circulation may be confined to libraries and dedicated investigators in the field. The period from receipt to publication of the articles is variable, often only comparable to that in established international journals, so that research workers may opt to present their more interesting findings to a wider readership rather than preach to the converted. □

J.M. Midgley is Professor of Pharmacy and Head of the Pharmaceutical Chemistry Division, Department of Pharmacy, University of Strathclyde.

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## Picture chemistry!

Peter Kollman

### Journal of Molecular Graphics.

Editor A.J. Morffew.  
Butterworth. 4/yr. £55, \$110.

THE *Journal of Molecular Graphics* (JMG), published in association with the Molecular Graphics Society, was founded to give "computational scientists" a forum to publish their work. The importance of computer graphics in studies of molecular systems, in drug design and in X-ray crystallographic studies of macromolecular structure has begun to be appreciated by many chemists. Indeed I am among the converts to the view that computer graphics is an essential tool for any chemist studying even moderately complex molecules. A corollary to this is that well-written computer software is often crucial in asking the most pertinent questions about a particular molecular system, and in answering them. Thus, there should be mechanisms for publication of software approaches to the solution of chemical problems and for giving due credit to elegant software.

In this context, it should be noted that

*Computers and Chemistry* (launched in 1976) and the *Journal of Computational Chemistry* (which first appeared in 1980 and was reviewed in *Nature* 299, 513; 1982) are journals where descriptions of both computer software and its applications are already welcomed. Of these two, it is significant that the latter has been the more successful because it has attracted a mix of software descriptions and applications of high quality and interest to many scientists.

In its first four issues, JMG has published articles mainly on computer graphics



algorithms. Sample applications of the algorithms often occupy a small part at the end of the article and typically the papers do not have a strong scientific punch-line. One cannot blame the authors for saving their scientific message for journals where more chemical scientists will read them, but in my opinion the separation of computer graphics from other areas of computational chemistry is not a good idea. Besides the regular articles, the journal has also included a bibliography of molecular graphics applications, abstracts from the meetings which include computer graphics, and descriptions of new computer products of potential interest to chemists.

One commendable advance made by JMG is to combine colour figures from the individual articles into a central section of colour, thus allowing high-quality colour pictures to be published without charge to the authors. The science itself can often be delivered most effectively with colour figures, yet most journals charge a large amount for publication (\$1,000-\$2,500 by the *Journal of Medicinal Chemistry*, *Journal of the American Chemical Society* or *Proceedings of the National Academy of Sciences*) and often also require colour-separated prints (for example the *Journal of Computational Chemistry*).

The appearance of this journal has certainly facilitated the publication of articles dealing with computer graphics, particularly in the approaches requiring examples in colour. Whether it will thrive will depend on many factors, principally whether the field of molecular graphics will emerge as a separate discipline or whether the journal can attract articles which appeal not only to the molecular graphics community but to a broader constituency of chemists. How the focus could be broadened is not clear, but the very title of the journal cannot help but give the impression that papers other than those dealing explicitly with graphics are less likely to be accepted or receive a wide readership. □

Peter Kollman is Professor of Pharmaceutical Chemistry in the School of Pharmacy, University of California at San Francisco.



## At the interfaces of spectroscopy

K.A. McLauchlan

### Computer Enhanced Spectroscopy: An International Journal.

Editor-in-chief H.A. Willis.

Wiley-Heyden. 4/yr. £65, \$130.

DATA-handling by computer and computer interfacing to laboratory equipment are very much to the fore in the 1980s, and the basic idea of a new journal to cover such matters is sound. *Computer Enhanced Spectroscopy* (CES) deals with any use of a computer in any branch of spectroscopy. Its contents include data manipulation on- and off-line, library searching, design principles of systems and specific experimental devices; unusually, much software is reported. Authors are invited to publish details which went undescribed in an original scientific paper, a seductive call to double their number of publications.

The problems for the journal, though, are several. Such a range of specialist spectroscopic techniques is involved that the matter dealing with any one topic is dilute. Since in many research fields there exist dedicated journals, it seems unlikely

that a seminal paper in, for example, magnetic resonance will make its way to CES. For ideas of inter-disciplinary interest, on the other hand, there exist the well-respected *Reviews of Scientific Instruments* and *Journal of Physics (E)*, which would take most of the material. Many of the practical accounts are device-orientated and concern specific microcomputers and specific types of spectrometer. They are of limited use, for wherever such interfacing is necessary one finds that because of technical advances many problems must be solved afresh. Similarly, software is usually most efficiently written for the precise microcomputer and application in mind, and often not in a high-level language. Some of the basic data manipulation points made in the four issues of Vol. 1 are very straightforward.

The journal is well and attractively produced with excellent reproductions of a wide range of devices, flow diagrams and software. However the first volume does appear to have been short of material, which has perhaps led to the publication of some rather trivial articles. Because of this uncertain start, and the reservations expressed above, I cannot believe that it will become widely read. □

K.A. McLauchlan is a Lecturer in the Physical Chemistry Laboratory, University of Oxford.

## Polymer exchange

Philip Hodge

### Reactive Polymers, Ion Exchangers, Sorbents.

Editor-in-chief F.G. Helfferich.

Elsevier Scientific. 8/yr. Dfl. 412, \$160.

FOR MOST of its history, polymer science has been mainly concerned with polymerization processes, the physical properties of polymers, and their applications in material science. There are many journals that cater for these areas. In contrast relatively little work has been carried out on synthetic reactive polymers. This is surprising, bearing in mind that nature makes extensive use of polymers in various recognition processes and to catalyse and control reactions between low molecular weight species.

In recent years interest in reactive polymers has increased considerably and *Reactive Polymers, Ion Exchangers, Sorbents* seeks to provide a forum for this

underdeveloped area of macromolecular science. The journal is well produced and contains original papers, short communications, reviews, book reviews, letters to the editor and a news page. For the first year it appeared quarterly but eight issues will appear in 1984, surely a sign of good health.

The standard of papers is excellent and there are contributions from many internationally known authors of good reputation. Thus far a rather high proportion of the papers has dealt with ion exchange resins, metal extraction using various sorbents and closely related topics. This bias probably reflects the fact that ion exchange resins are one of the few types of reactive polymers which have been studied for many years and have industrial applications. There are, however, many other areas of interest — polymer-supported catalysts, chemical modifications of synthetic and natural polymers, and biomedical and pharmaceutical applications, for example — and it is to be hoped that the number of papers dealing with these areas will increase to give the journal a better balance.

It is still hard to say how well this journal will fare in the long term. It serves a growing area of polymer science and if it can attract sufficient papers and publish them more frequently, whilst maintaining its present quality, it could do well. □

Philip Hodge is a Reader in Organic Chemistry at the University of Lancaster.

## Separate ways

Alwyn Mc Kay

### Solvent Extraction and Ion Exchange.

Editors E. Philip Horwitz and James D. Navratil.

Dekker. 6/yr. \$150 (US), \$166.50 (elsewhere).

THE launching of *Solvent Extraction and Ion Exchange* (SEIE) may well be considered overdue, in view of the enormous expansion in this area since 1945. The journal is a companion to *Separation Science and Technology*, and its appearance follows a questionnaire addressed to specialists in the two related fields.

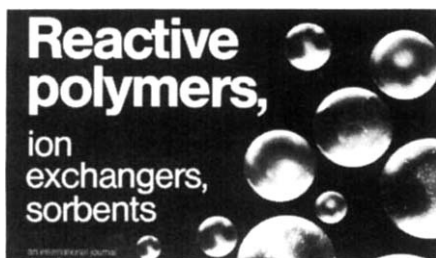
The first volume of four numbers, published during 1983, contains important material. There are 32 papers, five "notes" (not obviously distinguishable from papers), two valuable review articles and two letters; a bibliography and other items are included in each issue. Volume 2 (1984) will be larger, with eight issues, and it is expected that the journal will be expanded yet further. Certainly, there is plenty of scope for this.

There is, however, a long way to go before the editors' hope of the journal becoming the principal vehicle for papers on solvent extraction and ion exchange can be realized. At present SEIE carries only a small proportion of the total of papers on the subject being published, as the number of entries in the bibliography demonstrates. Moreover the selection is unbalanced in various ways. Academic chemical topics predominate, with emphasis on matters of nuclear interest, and there is little on equipment or industrial processes. On the other hand, the ratio of four solvent extraction papers to one ion exchange paper seems a fair one.

The journal is reproduced directly from the authors' typescripts and diagrams, but despite this it is not particularly cheap. The quality of presentation varies from very good to just adequate; in the worst cases one wishes that the method of printing did not preclude sub-editing to get rid of misprints, poor English and other blemishes. On the positive side, photographic reproduction should speed up publication, and most papers do appear reasonably promptly. The bibliography, however, being based on *Chemical Abstracts* rather than current journal contents lists, is unfortunately somewhat out-of-date.

My verdict is that SEIE indeed fills a gap. But there is some sacrifice of quality to economy and speed of production, and the editors would do well to keep an eye on their standards. □

Alwyn McKay was formerly a Group Leader in the Chemical Technology Division of the Atomic Energy Research Establishment, Harwell, Oxfordshire.



## The carbohydrate competition

Neil Baggett

**Journal of Carbohydrate Chemistry.**  
Editor Donald E. Kiely.  
*Dekker.* 4/yr.  
\$110 (US), \$121 (elsewhere).

THE aim of the *Journal of Carbohydrate Chemistry* (JCC), which in part replaces the *Journal of Carbohydrates, Nucleosides, Nucleotides*, is to provide an international forum for aspects of carbohydrate chemistry, excluding polysaccharide studies. This area of chemistry is already served by *Carbohydrate Research*, so that JCC does not fill a vacant niche but competes with the established journal. It is therefore appropriate to compare the two.

The new journal includes original research papers and communications, with some reviews of "timely topics in carbohydrate chemistry". *Carbohydrate Research* does not contain reviews but does cover studies on polysaccharides. Both journals publish articles in English (mainly), but also in French and German. The new journal comprises four issues per year (six were originally promised), so it is much smaller but also much cheaper than its competitor; the two are roughly comparable in terms of price per page. Perhaps the most noticeable difference between the journals is that JCC is repro-

duced direct from the authors' typescripts, which means that very rapid publication can be achieved but also, on occasions, that minor but necessary editing is not done. Together with the non-uniformity of type, this gives the journal a rather unfinished look, and the principal attraction of JCC for potential authors must be the speed of publication.

The claim to be an international journal is justified. There are representatives from 13 countries on the editorial board and the papers published to date originate from a wide geographical range. There is, however, a bias towards the United States, with almost half of the editorial board and one-third of papers coming from that country.

So far the standard of papers and reviews in the new journal is generally good, perhaps because roughly half of them have been contributed by members of the editorial board. The viability of JCC will depend on whether papers of a similar quality are forthcoming in the future from a broader range of authors, especially if the American Chemical Society continues to expand its range of publications into the area of carbohydrate chemistry. Whilst there are advantages for authors in having two journals to choose between, librarians are not in such a happy position — the standard of the papers in both JCC and *Carbohydrate Research* means that neither can be just ignored. □

Neil Baggett is a Lecturer in the Department of Chemistry, University of Birmingham, UK.

## Nucleic acid news

Janet L. Rideout

**Nucleosides & Nucleotides.**  
Executive editor John A. Secrist III.  
*Dekker.* 5/yr. \$149.50 (US),  
\$163.25 (elsewhere).

*Nucleosides and Nucleotides* (NN) is unashamedly a speciality journal, but one which covers an especially vigorous area of science. Its aim is to concentrate upon the chemistry and biology of nucleosides, nucleotides and closely related compounds, with the chemistry of new entities being emphasized.

Obviously, for this, as for most new journals, success in the long run will depend upon the submission of material from authors who generally send their papers to more established publications. Judging by the eminence of some of the contributors to the first two volumes, NN has made an auspicious beginning. And although the journal is reproduced from camera-ready copy, the standard of production is very high — reviews, original articles and notes are clearly presented, with chemical structures, tables and so on embedded as appropriate in the text.

Submissions are refereed, and must contain experimental details (a refreshing feature to be recommended to the editors of other journals devoted to the rapid publication of scientific research). Authors of review articles are encouraged to include critical comments, and thus far reviewers are responding and are doing more than simply providing compilations of the literature pertaining to a given subject. Lately, abstracts of recent articles and biblio-

### Nucleosides & Nucleotides

graphical data on selected subjects have been added to the journal's contents.

Anyone working in this field, or wishing to enter it, will need to refer to NN for information on developments in the chemistry and biology of nucleic acids, for indications of new directions the field is taking, as well as for the comprehensive reviews which appear from time to time. The subscription price is not unreasonable, and altogether the journal seems set for a healthy future. □

Janet L. Rideout is a Group Leader in the Organic Chemistry Department at Burroughs Wellcome, Research Triangle Park, North Carolina.

## With the grain

Donald D. Kasarda

**Journal of Cereal Science.**  
Editor T. Galliard.  
*Academic.* 4/yr. £43, \$98.

BECAUSE of the enormous importance of cereal grains as food, animal feeds and raw materials, almost every cereal-producing country in the world — from Australia to the USSR — has in recent years made huge investments in cereals research. Such expansion has created a need for extra journals as outlets for the resulting publications. The new *Journal of Cereal Science* (JCS) covers much the same areas as the already well-established journal *Cereal Chemistry*, including research papers (and an occasional review) on topics such as component characterization, nutritional quality, food science and technology, and genetics as they relate to the functional or nutritional quality of cereal grains (unlike *Cereal Chemistry*, however, JCS does not accept papers on legume "grains").

I have the impression that, in addition to a need for more journal space, the impetus for the formation of JCS stemmed partly from dissatisfaction by some scientists (particularly in Britain and France) with what they considered unfortunate reviewing practices by *Cereal Chemistry* that delayed publication of papers. They were also unhappy with the page charges levied by that journal. Since then, it should be said, *Cereal Chemistry* has dealt with many of its problems although the page charges remain.

My examination of five of the six issues out at the time of writing tells me that JCS is doing well; many papers are of a high standard, the quality of editing and of production are good, publication occurs a not-unreasonable six to ten months after receipt of a manuscript, and there are no page charges.

A few papers seem rather pedestrian, however, and such contributions may discourage authors from choosing this journal to publish their better work. On the other hand, authors must contend with the tendency of editors of the better general-science journals to recommend that papers dealing with cereal grains, even reasonably good ones, should be published in speciality journals. Such attitudes, however unfortunate, benefit *Cereal Chemistry* and now, I suspect, JCS as well.

I believe this new journal is here to stay. Not only that, the commitment to improved quality evident in occasional editorials by the editor T. Galliard, and one of his colleagues, J. D. Schofield, should lead to expansion. □

Donald D. Kasarda is Research Leader of the Food Proteins Research Unit, Western Regional Research Center, ARS, US Department of Agriculture, Albany, California.



## Agricultural produce

Leonard Broadbent

### Crop Protection: An International Journal of Pest, Disease and Weed Control.

Editor G.E. Russell.  
Butterworth. 4/yr. £68, \$136.

### Crop Research.

Editors C.A. Wood, Pauline B. Topham, Jane Bryan-Jones and P.J. Dudney.  
Scottish Academic Press.  
2/yr. £13.50, \$30.

### Biological Agriculture & Horticulture An International Journal.

Editor R.D. Hodges.  
A B Academic Publishers, PO Box 97,  
Berkhamsted, Herts HP4 2PX, UK.  
4/yr. £19, \$33 (personal); £55, \$89.50  
(institutional).

HORTICULTURE and, in historical terms, its offshoot agriculture, are multi-disciplinary technologies developed during the past 12,000 years. Much more recent is the science-based but energy-intensive production of plants and animals, to which are devoted so many journals. The three well-produced publications reviewed here add to this literature but do not fill gaps in it.

*Crop Protection* (CP), published since 1982, is attracting good papers from all over the world. An analysis of the four numbers available for review gives 15 papers from Britain, 12 from the United States and 20 from ten other countries, covering every aspect of protection science and practice, including biological, chemical and agronomic control, plant breeding, engineering and economics. It has six well-known and expert editors, and a distinguished advisory board of 15 members drawn from almost as many countries — presumably as bait, as possibly was the initial subscription of £45, which in two years has risen to £68. For this one gets about 48 scientific papers or reviews per volume, averaging ten pages in length, that is, a cost of about £0.14 per page of scientific paper (not counting book reviews). This is expensive: an old-established journal publishes similar papers, with more words per page, profitably at £0.07 per page.

*Crop Research* (CR) is not new but continues *Horticultural Research*, and remains a journal for the publication of research by the Scottish Crop Research Institute. About half of the papers originate there, and a few are from countries other than Britain. As one would expect from an institute with a good reputation, most of the papers are of high standard, but an editorial board of 17 and five associate editors seems over-weight for an average of ten papers per year; indeed, it has always been difficult to justify the existence of this publication when there are several long-established journals covering the same

areas. The advantage of CR is of quick publication, some papers taking only 6–8 weeks from receipt, although the average is nearer 28 weeks (about the same as for CP and for other refereed journals). The cost per page of scientific paper is £0.12 (1984 subscription rate) which again is expensive.

There are many who believe that “biological” or eco-agriculture is distinct from (and better than) “conventional” farming because of its holistic approach. The new journal *Biological Agriculture & Horticulture* (BAH) was started in 1982 by the then seven-year-old International Institute of Biological Husbandry, with the aim of publishing scientifically sound research and reviews “to raise the credibility of the biological system”. That this is necessary may reflect sadly upon the blinkered outlook of so many “academic” scientists; on the other hand, applied biologists in agricultural research have long realized the necessity for an ecological approach, and have been publishing sound epidemiological research results for decades.

Eco-agriculture has not, in general, had a good press, because of the tendency of some of its supporters to peddle prejudice as solid scientific fact. The editors of BAH will have to take care that their journal does

not follow the precedent, for 58% of the scientific content of the first five numbers consists of reviews and surveys. These, and the 14 papers reporting scientific results, come from ten countries, so the journal justifies its claim to be international. The cost of Vol.2 (1984) is not cheap at £0.16 per small page (if the size remains the

## BIOLOGICAL AGRICULTURE & HORTICULTURE

same). Four editors are again “advised” by a prestigious board of 23 members, drawn from seven countries. Being a journal of a society it contains conference reports and news, as well as book reviews and notices.

There is no doubt that food and fibre production will remain man’s greatest challenge for many generations. So there should be ample opportunity for BAH to improve its standing amongst agronomists and scientists in the future. □

Leonard Broadbent is Emeritus Professor of Biology and Horticulture in the University of Bath.

## Relative value

Julian Davies

### Trends in Biotechnology.

Editor John Hodgson.  
Elsevier (Cambridge, UK). 12/yr. £28,  
\$49, Dfl. 130 (personal); \$165, Dfl. 430  
(institutional).

### Bio/Technology: The International Monthly for Industrial Biology.

Editor Douglas K. McCormick.  
Nature Publishing. 12/yr. \$78, £70.

FOLLOWING the spate of four-page rumour sheets which accompanied the biotechnology industry in its headlong rush to establish a foothold in the world of commerce, publishers have decided to signal the consolidation of the industry by providing more substantial reading matter for the biotechnologist. In the spring of 1983 Macmillan launched *Bio/Technology* (BT) and Elsevier presented *Trends in Biotech-*



*nology* (TBT), both of them intended to create and to meet the need for reliable, intellectual presentations of modern biotechnology. A stablemate of *Nature*, BT will obviously benefit from the association, while TBT is fashioned along the same lines as the excellent *Trends in Biochemistry* (TIBS), a monthly collection of reviews that has a good following in academic circles. The nickname TIBS is well known

and a similar mnemonic for TBT would have been a definite advantage. What can we call it? TIBOTS? TOBS? TIBITS?

These newcomers to the biotechnology arena offer higher credibility and scientific acceptability than the other publications presently available in this field (with the exception of *Biofutur*; BT is actually more of an English cousin of this French biotechnology journal than a relative of *Nature*). But their serious approach apart, the two journals are quite different in format, presentation and goals.

TBT concentrates on up-to-date reviews of various aspects and developments in biotechnology and does not concern itself with commercial issues. It is essentially academic and pedagogical in character. By contrast, BT provides much information and comment on issues to do with the regulation, investment, business and international aspects of biotechnology and the products so eagerly anticipated. No other publication provides quite the same scope, though there is some overlap with *Nature* in the presentations of the health of the biotechnology industry. BT also includes a rather perfunctory and generally useless summary of new patents which seems to do nothing more than fill space; other patent review journals such as *Derwent Biotechnology Abstracts* and *Telegen Reporter* do the same thing much better.

Both BT and TBT carry substantial advertising. BT has more glossy advertisements than most journals whereas TBT tends to be more conservative. One infuriating aspect of BT is that the longer and better review articles are often interrupted by as many as six pages of advertising, and

in some issues the pages of advertising outnumber the pages of text. However, it is comforting to know that the two journals are well-supported in this way.

Both BT and TBT try to address immediate and future problems of biotechnology in the form of reviews. In recent issues, TBT has carried perceptive articles on delivery systems for drugs and enzyme engineering while BT has presented surveys of novel bioreactor systems and the factors involved in the design of pilot plants. Both



have good book reviews and are developing interesting "Letters to the Editor" sections. BT includes two or three original scientific research articles in each issue. The articles are related to recombinant DNA applications and are usually of good quality — more or less equivalent to the type of paper published in *Nature*. In fact they probably could be published in *Nature*, were it not for the fact that this would remove one of the justifications for the existence of BT!

One cannot finish discussion of BT without mention of Bernard Dixon, who is clearly one of the more potent commentators on the science/business scene. His articles are sometimes pungent, usually provocative and always worth reading. Perhaps it is in this respect that BT has the edge over TBT — it invites controversy. Yet the question whether the more academic approach of TBT will win and maintain the necessary readership seems to have been answered already; published six times a year until now, it will go monthly in 1985.

The bottom line comes with the question, are these two journals needed? Indispensability is always hard to justify but BT and TBT have quite obviously created a market for themselves. They are clear advances on the rumour sheets and are likely to be the best media for presentations of the science of biotechnology and its academic and commercial implications. The fact that both of them have distinguished editorial boards to oversee their content and shape their future should guarantee their continued quality in a field of publication where quality is rare. □

*Julian Davies is President and Research Director of Biogen SA, Geneva.*

### Journal prices

Details of editors and frequency of publication, and the annual subscription rates appearing at the top of each review are given in most instances for 1985. These details are not complete in all cases, and readers interested in subscribing to a particular journal should check the rates with the publisher concerned.

## Common ground for new biologists

Bernard G. Forget

### Molecular Biology & Medicine.

Editors E.S. Lennox and S. Brenner.  
*Academic.* 6/yr.

TO QUOTE its editors, *Molecular Biology & Medicine* (MBM)

takes as its main task the provision of a meeting ground between the basic scientist and the clinician, and to build up connections between molecular biology and clinical medicine.

The focus is therefore on normal and abnormal human biology and related research topics in the disciplines of molecular genetics, cell biology, immunology and biochemistry.

In the first five issues, the subjects that have been particularly emphasized include immunology-related research, ranging from studies of monoclonal antibodies (of human as well as murine origin) to analyses of immunoglobulin and histocompatibility genes; molecular genetics of haemoglobinopathies (thalassaemia and sickle cell anaemia); structural analysis of human viruses; and applications of restriction fragment length polymorphisms (RFLPs) to the study of human genetic disorders. The subject matter of MBM is therefore very broadly based and should be of interest to investigators in a large number of different fields. The article thus far published have originated from leading research laboratories, and although the specific reports have not generally dealt with findings worthy of comment in (say) *Nature's* News and Views section, they nevertheless constitute observations of genuine significance.

In addition to research papers (both long and short), the journal also publishes concise reviews of topical subjects — DNA-based prenatal diagnosis of haemoglobin disorders, chromosome translocations associated with leukaemia, X chromosome inactivation, the relationship of growth factors to cancer, and the association between polymorphisms in the major histocompatibility complex and different disease states, are among the topics covered in the early issues. Meeting reports and book reviews are planned but none have yet appeared.

The editorial in the first issue stressed the importance of rapid publication in the fast-moving fields covered by MBM. However this is an aim that does not appear to have been met. A bimonthly publication schedule was planned, but the most recent issue of the journal, dated December 1983, became available only in August 1984 and contained papers received up to 12 March 1984; the cover date therefore does not accurately reflect the actual publication date.

This journal has a very worthwhile

purpose in trying to establish a forum where the basic scientist and the clinician can find common cause; the scope and quality of the papers in the first issues give promise that this goal will be achieved. Overall, MBM will be a very useful addition to the libraries of individuals interested in keeping up to date on advances in the elucidation of the pathogenesis of human diseases as studied by the research technologies of the new biology. □

*Bernard G. Forget is Professor of Medicine and Human Genetics at Yale University School of Medicine.*

## Records of cancer control

Tony Davies

### Cancer Investigation.

Editor-in-chief Yashar Hirshaut.  
*Dekker.* 6/yr. \$95 (US),  
\$111.50 (elsewhere).

### Journal of Biological Response Modifiers.

Editor-in-chief Robert K. Oldham.  
*Raven.* 6/yr. \$80 (personal);  
\$105 (institutional).

### Clinical & Experimental Metastasis.

Editors K. Hellman, G. Nicolson,  
L. Milas and S.A. Eccles.  
*Taylor & Francis.* 4/yr. £49, \$98.

THESE three journals have in common an emphasis on cancer research, two of them obviously so from their titles and the third, covering biological response modifiers, because of its strong affiliation to a programme of the same name which is part of the (American) National Cancer Institute research scheme.

*Cancer Investigation* (CI) is an all-American effort in that all of its editorial and advisory staff work in institutes in the United States, about 30% of them in the Memorial Sloan-Kettering Cancer Center. The papers reflect this bias fairly accurately. The format is glossy, the illustrations of high quality. There is a deliberate attempt by the editors to include a proportion of review articles both on basic and clinical science, while controversies to do with clinical management are given an airing. For example there is a well-argued account of the virtues or lack thereof of staging laparotomy in Hodgkin's disease, and an article strongly critical of the current practice in the United States of performing mastectomy on the majority of breast cancer patients. Also evident are some pointers to technological advances.

The journal is intended, it seems, to provide a lively account of cancer research for a predominantly clinical readership. The miniseries (*sic*) on oncogenes and



cancer led me to believe that here was a splendid archaism, but on reflection I realized that there had been a further enrichment of the English language because of the elision of a hyphen. The editorials are rather pompous and turgid, and censorious of a government which dares to restrict the amount of money put into cancer research. It is not possible to determine how publication time relates to receipt of papers nor is it clear whether or not there is a refereeing system.

The cover of CI displays "Cancer" in big white letters, like its much older antecedent of that name. "Investigation" is added in much smaller black type. As few such journals are likely to attract the casual buyer, such a ploy could hardly be thought deceitful but to a disinterested outsider it seems misleading.

It has always been difficult to define "biological reagents", and the concept of a biological response modifier, apparently coined by Dr Vincent T. DeVita Jr, Director of the National Cancer Institute, is even more complicated. As stated in the *Journal of Biological Response Modifiers* (JBRM), it includes consideration of

a large number of agents: immunoaugmenting, immunomodulating, and immunorestorative agents; interferon and interferon inducers; lymphokines; cytokines; thymic factors; antitumor and antilymphoid cell antibodies; tumor antigens and modifiers of tumor antigens on cell membranes; antigrowth factors; and maturation and differentiation factors.

Essentially, it means what the Biological Response Modifiers Programme Coordinators want it to mean and anything else for which there is almost a splendid, albeit fictional, precedent. The large editorial board has one or two lonely foreigners known to be sympathetic to the Programme, but the JBRM is so far also predominantly an American effort in



content. The argument is advanced, quite fairly, that pressure on other journals makes the new journal inevitable in the welter of "biologicals" emerging from the monoclonal and genetic engineering technological revolutions. Again, no indication is given of publication times.

There are published here opinions on contentious issues in science. For example, Harold Hewitt is given the opportunity to air his well-known opposition to the tumour immunology package and in a subsequent issue Ronald B. Herberman fires back a broadside against such temerity. The papers make lively reading if one can swallow an initial anxiety at the rag-bag of topics. If the contributions to

JBRM are well refereed the journal could prove to be a useful addition to the literature, and might even act as a quality control device on the burgeoning "biologicals" industry. But it is, I fear, a crusading effort with little chance that impartiality and objectivity will count for much.

K. Hellman and G. Nicolson are prominent members of that part of the scientific community which gives special consideration to the metastasis of cancer cells. Now they have started their own journal, *Clinical & Experimental Metastasis* (CEM). There are others on the same topic but this has not proved a

## CLINICAL & EXPERIMENTAL METASTASIS

deterrent. A deliberate effort has been taken to make the journal fully international, in that there are representatives on the editorial board from each of a number of countries in which cancer research is funded. The papers published reflect this international flavour. As yet, CEM contains no frills in terms of the airing of controversies, letters to the editor and the like. The illustrations are good, the papers readable and informative. The publication time is difficult to determine on the first few issues but it seems to be coming down from a year or so to about four to six months. It is implied that there is a refereeing system, the submission times being given separately from a time of acceptance. I find it difficult to understand why journals do not state explicitly what their policy is on this important matter. Much of the work of scientists is judged by their output of papers, which in the good old days could reasonably be assumed to be subject to the regulatory influence of studious and independent refereeing. With such a plethora of new journals it is not easy to know if standards are being upheld.

There is little doubt that the technological advances of the past few years will, in time, enable us to control malignant cells in the human body (though the difficulties involved should not be underestimated). All three of the journals will contain a part of the record of progress and they are welcome on this score. But to my mind, well before the story is anywhere near complete, the profligate use of trees to act as the vehicle for scientific pronouncements will have ceased and new information will be surveyed on a television screen. It would have been good to have seen at least a hint that these new journals were preparing for the likely change in medium. □

Tony Davies is Chairman of the Section of Biology at the Institute of Cancer Research, London.

## Short of practice in immunology

Gordon Reeves

### Diagnostic Immunology.

Editor Mariano F. La Via.

Alan R. Liss. 4/yr. \$45 (personal); \$90 (institutional).

THE immunological literature is both extensive and fabulous. The clinical immunologist has the dual task of keeping abreast of advances in immunobiology, immunochemistry, immunogenetics and immunopathology, and of assessing their relevance to the study of human disease. The first requirement is well served by the excellent *Annual Review of Immunology* and *Immunology Today*. But what of the practice of clinical immunology? Of the various journals which purport to cover this end of the spectrum, only *Clinical and Experimental Immunology*, in my view, maintains a consistently high standard. Nevertheless, the need for a journal which seeks to improve the quality and organization of current practice, as identified in the editorial for the first issue of *Diagnostic Immunology* (DI), is probably a real one.

Unfortunately, little of the content of the first four issues of DI concerns current clinical practice. Although the pitch is often research-and-development orientated, much of this material compares unfavourably with papers appearing in the well-established *Journal of Immunological Methods*. Two useful reviews have appeared on clinical applications of FACS techniques and immunodiagnosis in protozoan and helminthic infections, but there has been little discussion of the practical value of existing methods and ways of delivering a clinical immunology service. Where are the critiques which review how to diagnose and monitor those disorders falling within the prime areas of clinical immunology, i.e. allergy, autoimmunity, immunodeficiency, lympho-proliferative disease and transplantation? The aim to provide "critical evaluation of reagents, quality assurance, standardization of methods and definition of normal ranges" has not been fulfilled. The emphasis of the third issue is on the "goal of providing a forum for discussion of new and promising methodologies", whereas the fifth issue (Vol. 2, No. 1) is the first one to contain a reasonable sprinkling of clinically relevant papers.

To me it seems that DI has undergone an identity crisis. Consistency of content, style and production are important prerequisites, if it is to succeed. □

Gordon Reeves is Consultant Immunologist and Senior Lecturer at the University Hospital, Queen's Medical Centre, Nottingham.

## Delving inside the cell

L.G. Lajtha

### Cell Biochemistry and Function.

Editor E.M. Crook.

Butterworth. 4/yr. Subscription rates for 1985 not available.

THE admirable editorial in the first issue of *Cell Biochemistry and Function* (CBF) indicates such a wide scope that it is difficult to say what precise niche the journal is attempting to fill. The aim appears to be to cater for studies of a biochemical nature on intact, whole cells. Certainly, although it is not specifically stated, the editors are obviously aware of the current emphasis on compartmental studies and of the biochemical challenges to be found in intracellular micromilieus and microanatomical structures.

An especially useful feature is the commissioned reviews, particularly the multi-authored ones which tackle a particular subject (for instance the five-paper review of mammalian alkaline phosphatase). Also included are short communications, full-length papers (with laudably detailed abstracts), conference reports, calendars of meetings, book reviews and, at the end of each volume, author and subject indexes.

The journal is nicely designed and produced — I particularly like the double-column presentation — but while the paper is of good quality, it does not give as much contrast to electron micrographs as one would like. It is truly an international publication, the papers coming from respectable laboratories all over the world, and the standard of contributions is high, both in science and in writing; the declared rights of the editor to make such editorial amendments as are deemed necessary does result in a pleasant uniformity and clarity of style.

I think that CBF will appeal to a wide

## CELL BIOCHEMISTRY AND FUNCTION

range of readers, although for four issues of about 65 pages each the subscription (£80 in 1984) is on the expensive side. The journal is a sound vehicle for studies of modern intracellular biochemistry and I suspect it will not remain a quarterly for long. □

L.G. Lajtha is Emeritus Professor of Experimental Oncology, University of Manchester.

## Thinking small

Richard W. Lacey

### Journal of Microbiological Methods.

Editor-in-chief D.C. White.

Elsevier Biomedical. 12/yr. Dfl. 510.

### Diagnostic Microbiology and Infectious Disease.

Editor-in-chief William J. Martin.

Elsevier Biomedical. 4/yr. \$132.

LIKE the cultures which so many of them discuss, journals on microbiological matters are proliferating. Do these two new contenders give readers (and authors) good value?

Each issue of the *Journal of Microbiological Methods* (JMM) contains about six substantial papers on aspects of the physiology, ecology and pathogenicity of bacteria (rather than of any other micro-organism). While the science is sound, most of the methods reported are specialized in scope and will appeal largely to workers in each specific field; so while libraries should stock the journal, there will be few individual subscribers. The text is readable — presumably reflecting high editorial standards — and the presentation is good; indeed it is not unlike that of the *Journal of General Microbiology*, to which some of the papers might have found their way. On the debit side, the photographs could perhaps be improved, and I wonder whether enough experimental details are given for the non-expert worker trying to reproduce the experiments.

To date most papers have been published within a few months of acceptance, but quite a number suffered delays due to revision. Was this because authors were not familiar with the journal's style, or did the editors demand improvements in presentation or in scientific content? Whatever the case, prospective authors would certainly not jeopardize their reputation by publication here.

I can be less positive about the stablemate of JMM, *Diagnostic Microbiology and Infectious Disease* (DMID). Each issue contains about eight full-length papers with a variable number of shorter contributions. Most papers are published

within six months of receipt, although here, too, many were revised. The standard of detailed editing seems to be high, and the presentation is again good.

The scientific content, however, is variable. The first issue of the journal (March 1983) contains a number of papers commenting on the antibacterial activity and sensitivity-testing of new antibiotics in arbitrary and uncritical ways. In them the objective in the synthesis of new antibiotics is apparently seen to be the identification of an agent with the broadest spectrum and lowest minimum inhibitory concentration. But surely the object of diagnostic microbiology should be rapid and accurate diagnosis, enabling the use of antibiotics with minimum selection pressure?

Contributions in later issues of DMID do fortunately manifest such an approach. Some of them could have been published in the *Journal of Infectious Disease*, and are of almost comparable standard, but it is difficult to see reasons for some work being undertaken, let alone published. For example, a paper in the January 1984 issue deals with *Pneumocystis carinii* infection in germ-free rats, concluding that "These data have significant implications for the natural history, diagnosis and epidemiology of *P. carinii* with regard to

## DIAGNOSTIC MICROBIOLOGY AND INFECTIOUS DISEASE

the human host". Such a comment is extremely disturbing, when it is appreciated how much the disease and therapy differ between the rodent and human being.

The journal seeks publications from clinical microbiologists and infectious disease specialists, but I remain unconvinced that members of either of these groups should deflect their energies in this direction, particularly at the expense of their clinical work. How many of our nosocomial infection problems are generated by neglect? Does our interest in novel antibiotics provide the need for these agents? □

Richard W. Lacey is a Professor in the Department of Microbiology, University of Leeds.

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## One of the places to get together

O.H. Petersen

### Clinical Physiology and Biochemistry.

Editors-in-chief J. Rosenthal and M. Rafelson.

Karger. 6/yr. DM 290, \$110.

AFTER a long time of ever-increasing specialization, resulting in smaller and smaller branches on the physiology tree, we now seem to have entered a period of reunification. One of the stated aims of *Clinical Physiology and Biochemistry* (CPB), "designed to provide a vehicle for scientific communications amongst clinicians, physiologists and biochemists", is "to re-emphasize those areas of common biologic interest that already exist". To some extent therefore it will be in competition with the older physiological journals, established before the fragmentation of the subject became a

### Clinical Physiology and Biochemistry

problem, of which at least *Pflügers Archiv* and the *Quarterly Journal of Experimental Physiology* deliberately take a broad view of physiology and include pathophysiological papers as long as they serve the purposes of the subject as a basic science.

Many of the papers so far published in CPB could, at least as far as the subject matter is concerned, have appeared in any of a number of physiological or biochemical journals. These papers are of a reasonable standard without being overexciting — as usual with a new journal only time will tell if it will be possible to attract really high-quality papers away from the broad-based journals covering the whole range of the physiological sciences, or from the journals covering the interface between the basic sciences and clinical investigations (such as the very successful *Journal of Clinical Investigation*), as well as from the many good multidisciplinary "systems" journals.

The editorial board appears to be somewhat narrowly based as an almost exclusive American-German enterprise, but CPB is the official journal of the National Academy of Clinical Biochemistry and presumably has a reasonably secure financial position. Only original papers are considered and there appears to be no wish to publish letters or notes. The delay from manuscript submission to publication seems long, particularly for a new journal; thus most of the papers in the July–August 1984 issue were received in August–September 1983. The quality of the production is good with

figures of uniform standard and the text set out in the now-usual two-column format.

The journal faces tough competition from a number of very different but well entrenched rivals. It will undoubtedly, as stated in the first editorial, serve as a forum to foster interdisciplinary exchange of information and concepts, but I am not so sure that it will become the major platform for this important exercise. □

O.H. Petersen is George Holt Professor of Physiology at the University of Liverpool.

## Infant alternatives

W.D.M. Paton

### ATLA (Alternatives to Laboratory Animals).

Editors Michael Balls, Rosemary Riddell and Alastair Worden.

FRAME, 5b The Poultry, Bank Place, Nottingham NG1 2JR, UK. 4/yr. £16.

ALTHOUGH labelled Volume 11, the issues of ATLA on which this review is based are indeed the manifestation of a new journal. The original venture, *ATLA Abstracts*, born in 1973, was not a success, probably because it was competing with more professional abstracting services, and perhaps also because it underestimated the extent to which biomedical scientists were already using non-animal methods. But the new journal, backed by an excellent editorial board, is a different proposition. Its policy (outlined in the first issue) remains that of its parent body, the Fund for the Replacement of Animals in Medical Experiments; but instead of abstracts, it includes research papers, general articles, correspondence, news, book reviews, and a list of titles covering all aspects of alternatives to laboratory animals in

biomedical research and toxicity testing.

Will the new journal find a niche? It deserves a chance. The news items are really valuable; nowhere else does one find, for instance, rational analysis of animal experiments from around the world, or compact accounts of the innumerable meetings now taking place. The research papers are mixed: some would hardly be published elsewhere, some are worthwhile. Although they would overlap with other journals, why not have a special place where (say) toxicity tests deliberately shaped to reduce animal use can readily be found? It is up to the editors to ensure the merit of such contributions, the only ultimate guarantee of their being read. The correspondence and book reviews could be equally useful. By contrast the melancholy, unorganized caravanserai of selected titles at the end of each issue seems useless; but again, if classified by technique, substance tested or general topic, with an author index, such a list could also be helpful.

The main difficulty for the journal however, may be strategic. One gets the sense of a battle being fought on two fronts: on the one hand a liking for verbal fisticuffs with the biomedical "establishment", of a type perhaps needful to retain support from the more anti-experiment clientele of FRAME; on the other a genuine scientific and critical approach to research, of the type winning respect from the investigator. Fisticuffs are quite fun, and rather encouraged these days, but are boring to re-read and do not wear well (except in the hands of a Housman, Leavis or Medawar). Dr Balls and his colleagues have a tricky task in child-rearing: one hopes that it is an occasional gentle irony (a tutorial with Lewis Thomas would do) along with some meat that finally clothes this promising infant. □

Sir William Paton retires this month as Professor of Pharmacology, University of Oxford.

## Science at play

E.C. Frederick

### Journal of Sports Sciences.

Editor Thomas Reilly.

E. & F.N. Spon. 3/yr. £39.50, \$84.50.

### Sports Medicine.

Editor-in-chief Rennie C. Heel.

ADIS Press, PO Box 34-030, Birkenhead, Auckland 10, New Zealand. 6/yr. \$95, SwFr. 210.

PUBLISHERS have responded slowly to the furious growth in sports science over the past decade. At a time when the number of researchers was doubling and tripling, the number of journal pages expanded in slow motion. As a consequence, long publication delays and abnormally high rejection rates became the norm.

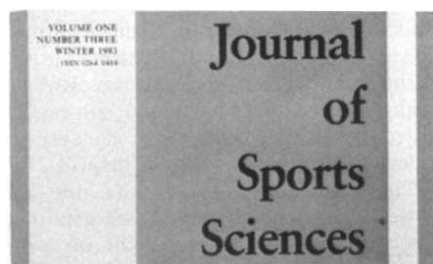
Although some of the pressure was

relieved by the introduction in 1980 of the *International Journal of Sports Medicine*, and by modest increases in issue size and frequency by many existing journals, it was not until the recent spate of new journals that sports science researchers finally found some breathing room. Two contenders for our papers and our subscriptions that should be mentioned in passing are the *International Journal of Sports Biomechanics* (which has just released its first issue and so it is too soon to gauge its scholarly impact), and *Human Movement Science*. The latter espouses an editorial policy encompassing most of the constituent disciplines of sports science, but up to this point has largely published motor-learning papers of somewhat specialist appeal.

The two newcomers that deserve more detailed description, and are reviewed here, are the *Journal of Sports Sciences* (JSS) and *Sports Medicine* (SM). The

editors of JSS have shaped their journal into a broadly based, multidisciplinary publication of consistently high standard. Each of the first four issues contains five to six full-length articles, mainly research reports, and an occasional review. In addition, book reviews and a comprehensive listing of forthcoming events appear at the end of each issue. Abstracts from the meetings of the Society of Sports Sciences, the journal's sponsoring body, are published at appropriate times.

Thomas Reilly and his colleagues have clearly avoided the danger of their journal becoming a place to recycle problem manuscripts — a pitfall that traps many



new journals — and the high rejection ratio of 70% of submitted manuscripts bespeaks the path they have taken to avoid the problem. No indication is given of the time required for review, but the median time from acceptance to publication is a quick two months.

In a field of science that is blossoming over such a range of subjects, it can require superhuman dedication to keep track of the trends in areas in which one is not active. *Sports Medicine* helps by publishing six issues annually, each of them containing review articles and "leading articles" which are a forum for the communication of key issues in sports science.

Although the journal's title suggests a clinical emphasis, the term sports medicine is used in a general, multidisciplinary sense; for example, articles in the first three issues cover topics as diverse as ergometry, biological rhythms, sports injury, bicarbonate ingestion, cycling physiology, sex and hereditary effects, and endorphins. The quality and depth of the contributions are uneven, as might be expected given such broad scope, but most are useful and well written.

JSS and SM are welcome additions to an area of science in need of deeper as well as broader journal coverage. These two well-executed newcomers help meet the need on both accounts. □

*E.C. Frederick is Director of the Nike Sport Research Laboratory, Exeter, New Hampshire.*

• A new book of interest to many researchers in sports science is Thomas A. McMahon's *Muscles, Reflexes, and Locomotion*. The book is published by Princeton University Press (price hbk \$50, £45.80; pbk \$15, £10.70), and will be reviewed in the 11 October issue of *Nature*.

## Eye to eyes

George Duncan

### Lens Research.

Editor-in-chief Sidney Lerman.

*Dekker. 4/yr. \$70 (US), \$81 (elsewhere).*

*Lens Research* has been launched upon a stormier sea than are most new journals. Not only do the usual financial stringencies confront it, but the very material that should provide much of the data for the journal — namely, the human lens — is, in many parts of the Western world, removed piecemeal in a messy homogenate rather than as a useful entity. The editor, however, takes a sanguine view of the latter difficulty and believes that it will lead to a rapid improvement in the range of *in vivo* techniques available for lens investigation.

The journal is produced by photo-offset and aimed at the rapid-publication market. It has a distinguished editorial board to steer it, and to set the tone many of them contributed to the first issue. The articles, all derived from a symposium held in Eindhoven in October 1982, are interesting and well produced. They deal with topics as diverse, but equally important, as the X-ray crystallographic structure of lens crystallins and the *in vivo* measurement of light-scattering from human lenses. However, a high proportion of the illustrations (I estimate almost 20%) in this

issue were reproduced from previous publications. This is an unwarranted intrusion in the first number of any journal, let alone one which purports to cater for the rapid publication market. (At present, it is in fact impossible to judge how quickly the submitted word is converted to the printed page, since neither submission nor acceptance dates are given in any of the issues so far produced.)

The production standard of the journal had slipped badly by the time the third issue appeared, since one article was published with an average print density of 6 words per line and 22 lines per page. I leave the reader to total up this page for comparison. Another article was photo-offset directly from a low-density dot matrix print-out; apart from contravening the published house rules for submission of articles, it was extremely difficult to read — a great pity in this particular case, since the paper described a good study carried out on those precious intact human cataractous lenses.

One most excellent feature of the journal was the inclusion of a comprehensive index at the end of the third issue. If this sort of care could be extended to the production side, and allowance made for comments on previously published articles, then it might be possible to foresee a calm and fruitful voyage ahead for this particular lens flagship. □

*George Duncan is Reader in Biophysics at the University of East Anglia.*

## Between two cultures

John Yudkin

### Nutrition and Behavior.

Editor Robin B. Kanarek.

*Alan R. Liss. 4/yr. \$35 (personal); \$70 (institutional).*

IT IS only recently that the traditional nutritionist, concerned with the isolation and description of the nutrients and their effects, began to recognize that the true scope of nutrition embraces the total relationship between people and their food. The subject is therefore based as much on the behavioural sciences as on the experimental sciences. The established journals of nutrition have started to reflect this by publishing occasional papers concerning such matters as food choice and nutrition education. Now comes *Nutrition and Behavior*, devoted wholly to such matters, with papers dealing with the effects of nutritional status on learning, memory and food choice, as well as with the effects of behaviour on nutrition.

A major problem is that those working in the laboratory sciences and those working in the behavioural sciences have developed attitudes and concepts that are often difficult to reconcile. The first paper

published in the first number of this new journal illustrates the point. Examining the relationship in children (aged from 5 to 16) between refined carbohydrate intake, hair cadmium and cognition, the authors measure food intake by the totally inadequate method of 24-hour recall. They also ignore the frequent demonstration by laboratory workers that hair analysis for mineral elements is quite unreliable. The tables and figures accompanying the paper contain no recognizable data such as the number of grams of carbohydrate and protein in the diet, or the scores of some of the intelligence tests, but are all derived after one or more mathematical transformations.

Later papers published in the four numbers of Vol. 1 of the journal will not be found so frustratingly incomprehensible; perhaps we can now expect the editors to be more critical of what is submitted to them. There is no point in providing a journal that, as they claim, is aimed at "interdisciplinary communication to researchers in a variety of fields" if what is published fails to communicate anything to most of its readers. Yet, if the journal does fulfil its promise, it will be an important contribution to the literature of nutrition.

*John Yudkin is Emeritus Professor of Nutrition in the University of London.*



## Divers advisers

Stuart Sutherland

### New Ideas in Psychology: An International Journal of Innovative Theory in Psychology.

Editors Pierre Moessinger, John Broughton and Richard Kitchener.  
*Pergamon*. 3/yr. £46, \$80.

PSYCHOLOGY is becoming a spectator sport. From the terraces a motley crowd of philosophers, linguists, sociologists, anthropologists and artificial intelligencers heckle, cheer and boo. But above all they proffer advice.

The first four issues of *New Ideas in Psychology* (NIP) contain a good many papers of this kind. Some authors advise psychologists to revert to teleological explanations, others urge them to seek salvation in artificial intelligence. Yet others recommend speculative theories provided they are testable, while perhaps the most original piece of advice is contained in a paper which advocates the founding of a science of happiness, to be known as eudology.

Almost all the papers are followed by

commentaries, a format that increases the interest of NIP, which is nothing if not eclectic. There are papers on such disparate topics as chimpanzee language, brief psychoanalytic therapy, personal construct theory and the existence of paranormal phenomena, and there is a solitary experimental report that suggests laughing gas operates through the orbital frontal cortex.

Although there is room for a psychological journal of speculation, one wonders whether the fields covered by NIP are not too diverse. Moreover, the quality of the contributions is a little uneven and one commentator's remarks on an article apply to several. He writes,

Like most programmatic manifestoes it serves the socio-psychological purpose of alerting like-minded scholars to the existence of a community of concern, but it fails to show signs of that rigor of analysis and systematic coverage of the subject matter that the author claims must distinguish a science.

The journal should provide an outlet for articles of this kind and will therefore doubtless serve an important "socio-psychological purpose". □

*Stuart Sutherland is Director of the Centre for Research on Perception and Cognition, University of Sussex.*

## Split psychology

Geoffrey Hall

### Journal of Comparative Psychology.

Editor Jerry Hirsch.  
*American Psychological Association*.  
4/yr. \$36 (US), \$39 (elsewhere).

### Behavioral Neuroscience.

Editor Richard F. Thompson.  
*American Psychological Association*.  
6/yr. \$100 (US), \$103 (elsewhere).

THE influence of the American Psychological Association is such that when it decides to reorganize its journals the international psychological community has to take notice. If a shift in policy does not reflect a change of emphasis in work in psychology, then it seems probable that one will result.

These two "new" journals are the fission products of the demise of the old *Journal of Comparative and Physiological Psychology* (JCPP) which existed from 1947 to 1982 as the continuation of an earlier (1921-1946) *Journal of Comparative Psychology* (JCP). The original JCP attempted to cover all aspects of the behaviour of non-human animals; its change of title recognized that physiological studies (of brain lesions and, latterly, of the effects of drugs) had come to predominate. When, in 1975, the other main line of work covered by JCPP (laboratory studies of conditioning and learning) was hived off as a special section of the *Journal of Experimental Psychology*, a reconsideration seemed

appropriate. What we have now been given in *Behavioral Neuroscience* is a journal devoted to "the broad field of biological bases of behavior" whereas the new version of JCP is concerned with "behavioral patterns... as they relate to evolution, development... and functional significance".

Contributors to a recent symposium on "the place of physiological psychology" saw in this split evidence of an identity crisis in their discipline. But in fact *Behavioral Neuroscience* with its sophisticated behavioural analyses of the effects of lesions and of drugs looks like a direct continuation of JCPP. The work reported is first-rate and this journal will retain its predecessor's reputation as being foremost in its field. JCP is something rather new but seems set to establish itself as a direct competitor to the respected ethological journal *Animal Behaviour*. Given the recent upsurge of interest in North America in what is sometimes called the "ecological" approach to animal behaviour, there is much good work to report and plenty of room for both journals.

These various divisions seem to follow natural fracture lines and are a sensible reaction to the growth of the field. Nonetheless, one looks back on the original JCP with a tinge of regret — ethologists have things of interest to say to learning theorists and both should communicate with physiological psychologists; it would be a pity if any new journal structure were to make such interactions less likely. □

*Geoffrey Hall is Senior Lecturer in Psychology at the University of York.*

## Nervous beginnings

A.G.E. Pearse

### Neurochemical Pathology.

Editor-in-chief Lloyd A. Horrocks.  
*Humana*. 4/yr. \$100.

CHEMICAL pathology, a term once popular, now has a decidedly antediluvian flavour, following absorption of the subject into clinical biochemistry. So what is neurochemical pathology except neuropathology in modern guise?

It comes as no surprise to learn that that is apparently just what the editors of this journal intended. According to their stated terms of reference, the journal is to "serve as the key forum for studies in the rapidly expanding area of biochemical neuropathology" and it "will thus be predominantly devoted to biochemical studies on all those tissues or body fluids that may help to elucidate the etiology or pathogenesis of neurological disorders".

*Neurochemical Pathology* is sponsored by the International Society for Neurochemistry and the World Federation of Neurology Research Groups on Neurochemistry and Cerebrospinal Fluid. I recognize, on the editorial board, many names derived from these two sources but I doubt if all were consulted in the matter of the creation of the new journal.

It may be pertinent to enquire, in so far as the first volume is concerned, just how far the editors have succeeded in their reasonably justifiable aims. There are 21 papers in the four numbers comprising the volume, and of these no less than 14 come into the category of experimental neuropathology. There are three purely neurochemical papers, each of which contains only a hint of future pathological importance, and three further papers describing chemical aspects of known, little known or unknown syndromes. The one remaining paper is an exercise in clinical neurochemistry.

With so great a preponderance of reports on experimental work — and despite the fact that much of it has some relevance, at least, to deficiencies or overdoses potentially occurring in human beings — one wonders whether the word "experimental" should not have been included in the journal's title. But it is clear enough that the editors did not intend their first year's coverage to present to the reader this predominantly experimental front, nor the virtual exclusion of several of their specifically cited areas of interest.

I conclude that several more years (more volumes) will require surveyance before it is possible to reach a positive conclusion that this journal is a useful one. Sad to say, with its present format and content it fails to justify its existence at all. □

*Anthony Pearse is Professor Emeritus of Histochemistry in the University of London.*

## Society's slice of neuroscience

R. Victoria Stirling

### International Journal of Developmental Neuroscience.

Editors-in-chief Antonia Vernadakis and Ezio Giacobini.

Pergamon. 6/yr. £86, \$150.

At its annual general meeting in 1983, the International Society for Developmental Neuroscience decided to break an affiliation with Karger, the publishers of *Developmental Neuroscience* (reviewed in *Nature* 293, 347; 1981), on the grounds of lack of copyright control, excessive page charges, long publication delays and poor illustrations. The new deal with Pergamon, who publish the *International Journal of Developmental Neuroscience* (IJDN) with the same editors-in-chief, seems much more advantageous. Subscription to the Society includes subscription to the journal, and the publisher also prints and distributes the Society's newsletter and collects membership dues. Members are exhorted to contribute to the new journal, the success of which should also accrue to the Society.

The instructions to authors are remarkably similar to those of *Neuroscience* (another society journal, under the editorial board of the International Brain Research Organization). But while the list of editors of IJDN spans many parts of the world, of 33 papers in four issues over half are from the United States, with a quarter

from Europe, three from Asia and one each from Venezuela and Finland (i.e. less cosmopolitan a range than *Neuroscience*). In the first issue of Vol. 2 are published an impressive series of abstracts from the Fourth International Congress held in Utah in 1983. I could find only one author who also contributed a paper to IJDN. Most of the contributions to the journal are biochemical in nature, very few being concerned with structural aspects of development. It is therefore difficult to judge the quality of photographic reproduction, but it seems reasonable.

The delay in publication appears to be



about six to seven months, though at the start of Vol. 2, the editors announced that to ensure a prompt service they had appointed associate editors in Venezuela and Japan; this was a good idea though the efficiency of the arrangement has yet to manifest itself. Apart from the minutes of the annual general meeting there is no editorial comment, although such a feature (and book reviews) had been suggested at the meeting.

This slim journal may potentially be a very useful publication for members of the Society. But unless they contribute meatier material, I cannot see it taking a larger slice of what is now a dwindling cake. □

R. Victoria Stirling is a member of the scientific staff at the National Institute for Medical Research, London.

## Trends in the risk business

J.R. Ravetz

### Risk Analysis: An International Journal.

Editor-in-chief Curtis Travis.

Plenum. 4/yr. \$90 (US), \$101 (elsewhere).

THE journal *Risk Analysis*, published by the Society for Risk Analysis, reflects the diversity of effort in this area, and also the history of the dominant trends. The attempts at "scientific" studies designed to determine the acceptability of technological risks began in earnest with a classic paper by Chauncey Starr in 1969. The failure of such attempts, first to model "low probability/high cost" hazards, and then also to quantify "acceptability", led to enrichment of the programme by behavioural studies during the 1970s.

The Society was formed rather late in the day, and in its earlier issues the journal still concentrated on a scientific approach. However, controversy soon broke out in its

pages; broader appreciation of the technological and environmental issues found expression in an increasing proportion of its contents. The founding editor, Robert Cumming, contributed to this development by his policy of soliciting articles and insightful editorials.

By now the journal presents a lively picture of current work, all the major articles being accompanied by invited critical comments. For instance, it has recently been the publication point for a seminal paper by Alice S. Whittemore, "Facts and Values in Risk Analysis for Environmental Toxicants" (Vol. 3, No. 1;

## Risk Analysis

1983). In this, it becomes clear that a new methodology, involving the complementarity of "facts and values" and of "knowledge and ignorance", is necessary if an effective discipline of risk analysis is to emerge. □

J.R. Ravetz is a Senior Fellow at Leeds University, and Co-Director of the SERC-ESRC "Risks" project.

## Developing forms of energy

Gordon MacKerron

### Renewable Sources of Energy: An International Journal.

Editor-in-chief Essam El-Hinnawi.

Tycooly International, 6 Crofton Terrace, Dun Laoghaire, Co. Dublin, Ireland. 4/yr. Subscription rates for 1985 not available.

ENERGY politics, and therefore energy studies, are bedevilled by an analytically damaging dichotomy between non-renewable and renewable sources. The split will, however, persist, and the latter have generally been served poorly. So there is a real need for a journal devoted to the renewables.

The principal aim of *Renewable Sources of Energy* (RSE) is to "facilitate the flow of information between developed and developing countries", with emphasis on the requirements of developing countries. While the central concern is ostensibly with energy technology, the journal contains a great deal on policy matters, particularly environmental control and local planning. In comparable existing journals (for example *Energy Policy* and *Energy: An International Journal*) renewables have received only sporadic coverage, while among publications dealing specifically with renewables, *Solar Energy* is narrower and more technical, and *World Solar Markets* and *Soft Energy Notes* are less academic. The focus of RSE is therefore unique.

The length of papers published to date has varied between 3 and 28 pages, each issue containing about 6 articles. There are, as yet, no editorials or comments on earlier papers, and only a rather perfunctory review section divided between "News and Views" and some short book reviews.

Articles generally fall into one of two types: semi-technical reportage of devices and projects (for example design optimization of solar water heaters; experience of a rural energy centre) and broader reviews covering such topics as geothermal energy or energy and community development in developing countries. The quality of the former is reasonably good, while that of the reviews is poorer, with some lapses into pious generalities.

Reflecting the editorial objectives, RSE's authorship is centred upon the developing countries, with a third of the early papers coming from India (and one welcome Chinese contribution). The main requirement in this field is for communication between developing countries and this journal should help to provide it. □

Gordon MacKerron is a Senior Fellow in the Energy Programme, Science Policy Research Unit, University of Sussex.



## Water works

T.R. Parsons

### Continental Shelf Research.

Editors Michael Collins and Richard W. Sternberg.

Pergamon. 6/yr. £63, \$110.

### Chinese Journal of Oceanology and Limnology.

Editor-in-chief C.K. Tseng.

Science Press, Beijing/Springer-Verlag. 2/yr. DM73, \$33.

TWENTY-FIVE years ago, oceanographers learned the terms "oceanic" and "neritic" with their vague implication of a discontinuity between plankton species (and therefore, presumably, the physical-chemical environment of the sea) as one approaches a coastline. Not until the past decade, however, has a general understanding emerged of the distinct oceanographic changes which occur as water flows over the continental shelf. Thus I am in complete agreement with Dr Sternberg's statement that research in this area has "come of age", and it is now helpful to think of many oceanographic processes as being either deep-sea or continental shelf phenomena. This is the rationale behind the appearance of *Continental Shelf Research* (CSR), which complements the longer-established journal *Deep-Sea Research*.

The new journal is interdisciplinary in aims and promises to meet a number of needs for oceanographers working on this specialized and, at times, highly variable environment. The first three volumes have contained a wide variety of papers on water movement, frontal zones, microbiology and geological processes. Although in the editorial policy it is not stated that the journal specifically excludes articles on pollution, the early issues are refreshingly devoted to what I would call the pure science of the oceans — studies on the use of the continental shelf as a dumping ground seem either not to have been submitted to date, or to have been excluded. The journal also contains short sections on instruments and methods, and book reviews. Written comments on papers will be considered for publication up to three months after the appearance of the original paper.

There are no Japanese oceanographers on the editorial advisory board and, in fact, one gains an impression from the choice of the associate editors that much of CSR may be given over to discussion of the margins of the Atlantic Ocean. To fulfil the avowed role of a "platform for international research programmes", the editors should have selected their assistants from a wider constituency. On the other hand, the interdisciplinary nature of the subject is reflected both by the composition of the editorial board and by the articles published so far. Clearly CSR has made a good

beginning. It should go on to become essential reading for oceanographers.

The Chinese-language journal *Oceanologia et Limnologia Sinica* was first published in 1957 and, by 1982, 410 articles on limnology and oceanology in China had appeared. The other journal reviewed here, the *Chinese Journal of Oceanology and Limnology* (CJOL) is not, as might be thought, a full translation of the Chinese journal. Authors who write in *Oceanologia et Limnologia Sinica* may have their work translated for publication in CJOL, but the journal will also accept articles from other Chinese-language publications as well as original papers written in Chinese. This, then, is how a country of one billion inhabitants and many new institutes of limnology and oceanology intends to present a representative sample of its science on these subjects to the English-speaking world.

Out of the first 23 articles published by CJOL, seven were concerned with physics, six with chemistry, two with geological processes and eight with biology; only two of these papers were limnological in nature. Geographically, most of the authors dealt with scientific problems within China, but one paper was concerned with the Tyne estuary in England.

The papers in physical oceanography do represent an up-to-date approach to the subject. The biological contributions, on the other hand, are mainly keyed to systematics and there is as yet little

indication of biological modelling or of more broadly based studies on descriptive ecology. In attempting to carry out their intention of covering a wide spectrum of aquatic science in China, the editors may have gone too far by including at least one paper in the first two numbers which is entirely physiological, while Part II of a paper in physics is promised for another issue — a promise which may be difficult to keep, given the vast amount of Chinese-language science which has to compete for space in the journal.

## CHINESE JOURNAL OF OCEANOLOGY AND LIMNOLOGY

中国海洋湖沼学报

In general, however, Professor Tseng and his colleagues have performed a necessary task in exposing Chinese limnology and oceanology to a wider audience. The journal is well produced, and the text gives little indication that it has been translated. But I have one plea. The editors should ensure that the location of each of the studies is identified. In many of the articles it is not; one example is the paper on the tidal equilibrium of Blue Bay — where in the world is Blue Bay? □

T.R. Parsons is a Professor in the Department of Oceanography at the University of British Columbia, Vancouver.

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# Geophysics: ins and outs of the library

Jean-Claude De Bremaecker

**Annales Geophysicae.** Editor-in-chief Stephan Mueller.  
Gauthier-Villars, 11 rue Gossin, F-92543 Montrouge Cedex, France.  
6/yr. FF780.

**First Break.** Editor Ian Williamson.  
Blackwell Scientific. 12/yr. £34.50, \$58.

GEOPHYSICALLY, Europe is slowly emerging from the deep: she wears a Latin dress but speaks English. At least, so it seems, for *Annales Geophysicae* (AG) has replaced both the French *Annales de Géophysique* and the Italian *Annali di Geofisica*.

As the official journal of the European Geophysical Society, the future of the journal is promising. It embraces the same subjects as its well-known American counterpart, the *Journal of Geophysical Research*; that is, all the fields covered by the International Union of Geodesy and Geophysics, but with one confusing exception: geochemistry and volcanology are not mentioned in the "scope of the journal". It appears that geodesy may also be excluded, but this is less certain. Further to confuse matters, the president of the EGS states in the first issue that volcanology and geochemistry as well as geodesy are *included*. The "scope" appears inside the front cover, the president's words on the facing right-hand page, and never have the twain met.

One presumes that all articles are refereed, and very speedily too, since the average delay for acceptance is about two months. The editorial board is made up exclusively of western European geophysicists, and very few contributors come from eastern Europe either, a geographical bias which should be corrected. The articles are of a remarkably high quality for a new publication, the abstracts not always so. Thirty-three years have passed since Kenneth Landes's classical "Scrutiny of the Abstract" (*Bull. Am. Ass. petrol. Geol.* 35, 1660) yet still one finds that "The theory...is developed. An algorithm ... is proposed. Examples...are presented. The possibility of application...is discussed". It seems only reasonable to hope that the editors will in future demand abstracts that are, in Landes's words, "a condensation of the essential qualities of the paper" and "not a mere recital of the subjects covered".

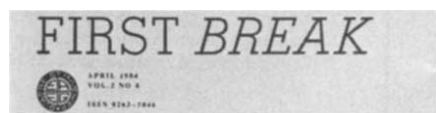
English, we all know, is a good simple international language (except for its spelling). As a non-native speaker residing in the United States, I particularly appreciate the problems which AG's authors and editors have faced and generally overcome. A simple example comes to mind: the French "Pour lever cette difficulté..." does *not* mean "in order to raise the difficulty". There are very few such examples in the journal, and perhaps the best solution to this problem is to forget

it; for, as scientists say, "the solution is not entirely clear", which means that it is utterly obscure.

One attractive feature of AG is the absence of page charges, coupled with the supply of 50 free reprints. The format (two-column on approximately 21 × 30 cm paper) is open and pleasant, the printing is of high quality, while the style and typography for the references are the best that I have ever come across. Much as in the *Journal of Geophysical Research*, only articles are published, but short notes are also welcome. So far no "comments" on previously published papers have appeared; presumably they will, eventually. Overall, at a subscription price of FF780 (c. \$84) outside France, this is a bargain that libraries should not miss.

*First Break* (FB) is a different sort of beast. It does not aim at being a scientific journal, instead publishing "short non-technical articles...and up-to-date news about the geophysics industry". It is thus not meant to be archived, but does fill a clear need by offering executives and others in the oil industry a means of keeping abreast of events. The industry news consists mostly of publicity releases, and has to be read with a dose of scepticism — that does not make it less valuable. It thus goes without saying that few libraries will want to subscribe to FB, which is to the good of everybody concerned.

The editor is to be commended for dealing exclusively with items connected with geophysics. This may seem obvious, but it is not. Only a little while back its counter-



part in the United States, *The Leading Edge*, was launched and unwisely included anything of conceivable interest to geophysicists. As a result its first numbers contained a mish-mash of right-wing propaganda, cross-word puzzles and sports news, with a dash of geophysics added for good measure. *First Break*, it seems, has profited from that sad example.

A common temptation with a publication such as this is for the publishers to try to "upgrade" it to a *bona fide* scientific journal. This shoal has not been avoided entirely by FB: at least one paper is reproduced from a well-known geophysical journal. Worse yet would be to fall to the level of taking non-refereed papers which have not been accepted (and could

not be) by established journals — far better to publish a slimmer issue than one containing second-rate material. One can only hope that the editor will keep his goal firmly in mind over the next few years; some of the papers are a little disquieting in this respect.

*First Break* is a readable, informative and reasonably cheap publication. But considering the world-wide scope of the geophysics industry and its currently rather depressed state, as well as the thinness of both FB and *The Leading Edge*, I cannot help but wonder whether these two publications should not merge. It might bruise some editorial egos but the readers of both might well be better served. □

Jean-Claude De Bremaecker is a Professor of Geophysics at Rice University, Texas.

## Talk of integration and communication

John Tarney

**Journal of Metamorphic Geology.**  
Editors M. Brown, T.P. Loomis and R.H. Vernon.

Blackwell Scientific. 4/yr.  
£47, \$97.50.

**Geochemistry.**  
Chairman of the editorial committee Tu Guangchi.  
Science Press, Beijing/Springer-Verlag.  
4/yr. DM 129, \$54.50.

IT WOULD not be exaggerating to say that well over half of the Earth's continental crust is composed of metamorphic rocks, so it is surprising that there has been no specific journal acting as a focal point for research in this area. Until now, observational details have been recorded separately in various national and international journals of mineralogy, petrology, geochemistry or structural geology, of which there are now a great many, and this has put limitations on the possibilities for an integrated approach to the subject.

The new *Journal of Metamorphic Geology* (JMG), titled as it is with the broad term "geology" rather than just "petrology" and so on, should provide an appropriate vehicle for the reporting of broader ranging research on metamorphic rocks, although this may be more commonly achieved through the publication of thematic issues. Certainly, detailed integrated studies are now beginning to tell us a great deal about the past thermal and tectonic histories of metamorphic rocks, and the importance of fluids, which may be of great value in attempts to reconstruct the geological evolution of a province in terms, for instance, of plate tectonics.

During its first year, JMG has attracted



contributions from an international group of authors on a wide variety of subjects, dealing with rocks from several continents which extend over an age span of 3,000 Myr. Admittedly, with one or two exceptions, most of these articles could have been published in one or other of the established journals without significant modification, so it remains to be seen whether the new journal will develop a character of its own: only time will tell, but the promise is there. The standard of presentation is high, the style conforms fairly closely to that of other journals published by Blackwell, and altogether JMG represents fairly good value for money.

*Geochemistry*, a Chinese journal which for the past two years has also appeared in an English-language version, is rather different. Of the several dozen articles which have appeared so far, almost all are written by Chinese authors (although one article has a non-Chinese co-author) and they deal almost entirely with Chinese geological and geochemical problems. Clearly, on the one hand, Chinese geochemists have accepted that English has become, for better or worse, the



international language in the geosciences; on the other, we see here implicitly their strong desire to participate fully in geochemical research at international level in future.

The range of topics covered is comprehensive — various aspects of igneous, metamorphic and sedimentary rock geochemistry, meteorites, marine geochemistry, economic geology and mineralization, organic geochemistry, isotopic dating, experimental studies and new instrumental techniques — and several articles on crystallography and new minerals have also been included. The scope, then, is wider than that of international geochemical journals, and extends into fields normally covered by journals of mineralogy, petrology and economic geology. The standard of presentation and the scientific content of the articles is generally high.

Now that several Chinese institutes have furnished themselves with a range of advanced geochemical instrumentation, comparable with that of any laboratory in the world, we can look forward to an increasing number of important contributions from Chinese geochemists. If that proves to be the case, *Geochemistry* will become necessary reading for many geoscientists. □

John Tarney is F.W. Bennett Professor of Geology at the University of Leicester.

## Heart of Gondwana

Paul Mohr

**Journal of African Earth Sciences.**

Editor-in-chief Cornelius A. Kogbe.  
*Pergamon. 4/yr. £49, \$90.*

AFRICA is no longer “the black continent with the big blank spaces on its map” — the description of Hans Cloos en route to his first employment as a geologist in German West Africa. Africa’s geology, mostly the preserve of European explorers before the First World War, has painstakingly become revealed through the work of colonial geological surveys and, especially since 1945, by an enthusiastic influx of European and American university personnel. Most of their work has been published in “international” (i.e. European and North American) journals.

Aside from geological survey reports, not notable as repositories for intellectual discussion and new ideas, the African geological literature has been scant (South Africa providing an outstanding exception). Now there appears the *Journal of African Earth Sciences* (JAES). Although published outside Africa, and with its chief and regional editors likewise resident outside the continent, it makes a bold beginning. African geologists and geophysicists have had increasing need for a publishing forum that transcends national boundaries yet keeps to the heart of Gondwana.

The format of JAES is African in its

spaciousness (21×30cm), it is well produced (the photographic plates are sharp, the line-drawings large and readable), and the print is a boon to eyes overly strained from reading some established journals. The first volume comprises 37 articles, not including book reviews and conference reports. The quality varies, generally in direct proportion to article length. Interestingly, Nigerian and Egyptian geological topics (there are no geophysical articles in this first volume) together make up more than half the volume. Only five articles deal with Francophone Africa, four of these in French. Southern Africa and Zaire are conspicuously unrepresented. Of the authors, about half are based at African institutions, which is a fair achievement.

Clearly the infant journal has some growing up to do before it can justify its continental scope and ambition. On the other hand, one notes that there is as yet no *Journal of European Earth Sciences*. Here, then, is a medium for the propagation of that natural entity, African earth science, and, more than that, for indigenous African geologists and geophysicists to develop their scientific mettle. On this first showing, JAES promises well and deserves every success. Its cost is not too unreasonable by wealthy European standards, but, ironically, it has priced itself out of the greater part of the African market it is designed to serve. □

Paul Mohr is Professor of Geology in the Department of Geology at University College Galway, Ireland.

## Birth in decay

Norman K. Grant

**Isotope Geoscience: An International Journal.**

Editor-in-chief G. Faure.  
*Elsevier Scientific. 4/yr. Dfl. 237.*

IF the distinction and international character of an editorial board are sufficient to ensure the success of a new journal, *Isotope Geoscience* (IG) should do well. The first six issues suggest that the editorial resources available to the editor-in-chief will lead to the publication of critically reviewed descriptive, theoretical and review papers of an attractively high standard. So far about two-thirds of the articles have been on the systematics of radiogenic isotopes, and about one-third on stable isotopes, the average time from manuscript acceptance to publication in the latest number being six to seven months. Book reviews are also published.

Elsevier has introduced IG into our libraries as a daughter journal to *Chemical Geology*. Whether the newcomer can meet its ambitious goals of fostering “scholarly communication between isotope

geoscientists of the world”, and “form a bridge” between them and other geoscientists remains to be seen. Isotope geoscientists are not a homogeneous group, and the range of interests which the journal proposes to serve is extremely wide — radiogenic and stable isotope and radiochemical systematics in inorganic and organic terrestrial and extraterrestrial materials.

It could be argued that the most fruitful approach for isotope geoscientists is to communicate first of all with scientists in complementary and converging disciplines. For this reason it seems likely that many of the most influential papers in isotope geoscience will continue to be published in established journals of a more general nature, such as *Contributions to Mineralogy and Petrology*, *Journal of Petrology* and *Earth and Planetary Science Letters*. If IG is read only by specialists its importance will be limited. So, for the time being, my advice to librarians is to place the highest priority on the purchase of the journal only when their institution includes staff actively engaged in isotopic research. □

Norman K. Grant is Professor of Geology at Miami University, Oxford, Ohio.

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## In the forecasting movement

Chi-Yu King

#### Earthquake Prediction Research.

Editor Tsuneji Rikitake.

Reidel. 4/yr. Dfl. 90, \$36 (personal);

Dfl. 180, \$72 (institutional).

EARTHQUAKE prediction, the scientific dream once shunned by respectable scientists, has been hotly pursued during the past two decades in such earthquake-prone countries as the USSR, Japan, China and the United States. Because of its interest to the public, and the multi-disciplinary nature of the research effort, many papers on the topic have been presented at a wide variety of scientific and non-scientific meetings, and published in many different journals and reports. Thus the appearance of *Earthquake Prediction Research* (EPR) to serve as a focal point for work on the subject is certainly very welcome.

The journal is published by a Japanese foundation (the Association for the Development of Earthquake Prediction) in cooperation with the Dutch publisher Reidel, with the objective of providing an international medium for original and review papers, reports and other items related to earthquake-prediction research. The editorial board, consisting of most of the current members of the Commission on Earthquake Prediction of the International Association of Seismology and Physics of the Earth's Interior, is strong and internationally well represented.

As of 31 July 1984, five issues of EPR had been published, three of them devoted to a single international symposium. All

# EPR

EARTHQUAKE PREDICTION RESEARCH Vol.2 No.1 1983  
 Edited by Tsuneji Rikitake

the issues arrived at our library in Menlo Park much later than scheduled. For example, Vol. 1, No. 1 (1982) and Vol. 2, No. 1 (1983), the last published issue, were received on 1 October 1982 and 9 November 1983, respectively. Thus the speed of publication is significantly less than one might infer from the manuscript receipt dates shown in the individual papers. Writing from personal experience, I submitted a manuscript on 1 November 1982, but was not informed of its acceptance until 9 June 1984.

Published in EPR so far are an introductory editorial, a meeting report and 29 "regular" papers, most of which are seismological (17) and rock-mechanical (9). About half of the papers are not directly relevant to earthquake prediction and could have been published elsewhere. Similarly, the scientific quality of the

papers ranges widely, from the mediocre to excellent. Some of the papers (from non-English speaking countries) could have done with additional editorial assistance. The quality of production is very good, comparable to *Pure and Applied Geophysics*, and the cost is reasonable (Vol. 1, which has 391 pages, cost \$69 for libraries and \$28 for individuals).

Having identified and filled a significant publication gap, EPR has a potential of becoming a fine international journal. Whether it will fulfil its promise depends on the ability of its editorial members to attract and promptly and properly process good, relevant papers from all the different disciplines concerned, including geology, geophysics, geochemistry, hydrology and biology. □

*Chi-Yu King is a Geophysicist at the US Geological Survey, Menlo Park, California.*

## Fusion across the fields

G.J. Pert

#### Laser and Particle Beams: Physics of High Energy Densities.

Editor-in-chief Heinrich Hora.

Cambridge University Press. 4/yr.

£50, \$135.

IN 1962, the observation of gas breakdown by high-power lasers initiated research into laser-generated plasmas as a possible source of thermonuclear power. However, before the introduction of the concept of near-isentropic compression in 1972 overcame the fundamental limitations, these proposals remained extremely speculative. It was shown that such compression could be generated by the ablation pressure of the blow-off plasma formed by surface heating of a simple deuterium-tritium pellet (sphere or shell). These initial designs have now been shown to be over-simple, in that the nature of the heating process may strongly modify the compression phase, and this problem particularly applies to laser-driven compression. As a result, ion-beam systems have been proposed as alternatives.

Over the past ten years the field has grown considerably, to the extent that probably about a quarter of the fusion effort around the world is now in this direction, and with a commensurate increase not only in number of workers but also in the range of activity. Research in this area now embraces nuclear burn and product studies, hydrodynamics, electromagnetic wave propagation, spectroscopy, and laser construction and design, in addition to the basic plasma physics. The field is thus rooted in several, distinct disciplines, which has led to



publication of research results in a wide range of journals.

Despite its somewhat misleading title, the object of *Laser and Particle Beams* (LPB) is to serve the inertial fusion and laser-plasma community. Although the creation of yet another contender for our dwindling library budgets may be questioned, this one must be welcomed; it covers a relatively new, multidisciplinary subject which is served by no existing journal.

The new journal is international in character, the editor-in-chief being Australian and the editorial board drawing

## LASER AND PARTICLE BEAMS

its members from Britain and the rest of Europe, Russia, the United States and Japan: this same wide distribution is also reflected in the contents. In the first volume, the standard of the papers is generally high, with contributions included or promised from many of the major laboratories, and they cover most of the range of the field. Publication time to date is relatively short (between six and nine months), and the journal is attractively presented, each issue containing 100 pages and about eight papers. No letters or research-notes are included, but there is a useful book review section.

The first issues show that LPB has made an encouraging start, but success in the future will depend on the editors' ability to attract the major papers at present published in a variety of specialist journals such as *Physics of Fluids*, *Journal of Applied Physics* and *Journal of Physics* Parts B and D. This new journal should become required reading for the inertial fusion community, and at its current subscription rate should be within the budget of most libraries. □

G.J. Pert is Professor in the Department of Applied Physics at the University of Hull.

### Autumn books

Nature's next review supplement is Autumn books, which appears on 15 November. The books to reviewed include:

- *Ideals and Realities*, edited by Z. Hassan and C.H. Lai, a collection of the essays of Abdus Salam.
- *The Limits of Science* by P.B. Medawar.
- *The 4th Dimension: Toward a Geometry of Higher Reality* by Rudy Rucker.
- *The Resourceful Earth*, Julian L. Simon and Herman Kahn's response to *Global 2000*.
- *Superforce: The Search for a Grand Unified Theory of Nature* by Paul Davies.
- *From Knowledge to Wisdom: A Revolution in the Aims and Methods of Science* by Nicholas Maxwell.
- The first three volumes in a new series, *Key Environments*, edited by John Treherne.
- *Discoverers of the Lost World*, George Gaylord Simpson's account of palaeontology in South America.

## Matter of the fourth state

R.A. Lawes

### Plasma Chemistry and Plasma Processing.

Editors Emil Pfender and Stan Vepřek.  
*Plenum*. 4/yr. \$95 (US), \$107 (elsewhere).

*Plasma Chemistry and Plasma Processing* (PCPP) attempts to cover "the fourth state of matter" — the plasma state — through the publication of research papers and articles on subjects such as plasma physics, fluid dynamics and heat transfer, surface chemistry, materials science and so on. The claim is made that the journal is a major international focal point for the scientific and technical community. It has yet to achieve that status.

The scope of PCPP is very broad and, with only about 20 papers published per annum, there is some evidence of a lack of focus. Viewed from a particular topic, for example plasma technology in microelectronics fabrication, only two or three papers may be of direct interest to an individual reader each year. Thus, for most

readers, this journal will be seen as complementary to other, more specific and established publications such as the *Journal of Vacuum Science and Technology*.

The quality of the papers themselves is good, with a strong emphasis on experimental results adequately supported by theory. Their source is world-wide (with a bias towards the United States!) yet firm editorial control has brought all contributions up to a consistent standard. General review papers are avoided and editorial comment is conspicuously absent. There appears to be little encouragement for readers to respond to previously published papers, and this is a failing which the editors would do well to rectify. The quality of production is uniformly excellent with clear text and legible, informative diagrams.

Broadly, PCPP has met most of its objectives as well as can be expected. At an export price of \$107 per volume it is probably a little too expensive to reside on personal bookshelves; however, research workers will wish to peruse the contents regularly and should persuade their libraries to take out a subscription. □

R.A. Lawes is Head of the SERC Electron Beam Lithography Facility at the Rutherford Appleton Laboratory, Chilton, Oxfordshire.

## Albite to zirconium

Robert Cahn

### Mechanics of Materials: An International Journal.

Editors S. Nemat-Nasser and J. Weertman.

*North-Holland*. 4/yr. Dfl. 252.

### Materials Chemistry and Physics: An International Journal.

Editor V. Lorenzelli.

*Elsevier Sequoia*. 12/yr. SwFr. 470, \$224.

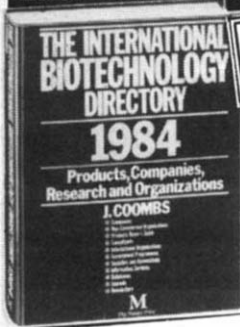
FLEDGLING engineers are taught a subject generally misnamed "strength of materials", which is mostly elasticity theory applied to the analysis of loaded components and structures. "Mechanics of materials", a more acceptable term, incorporates some of this, but much else besides: it includes the study of plastic flow, fracture, fatigue, the mechanical characteristics of cracks and other precisely defined defects, the mechanics of powders and other porous media, mechanical anisotropy and more. *Mechanics of Materials* (MM) is also the title of a new journal which has got off to a flying start since its first appearance early in 1982, and now publishes some 400 pages each year. Its subheading specifies that it is a "forum for theoretical, experimental, and field advances in mechanics of flow, fracture, and general constitutive behaviour of geophysical, geotechnical and technological materials".

The geological emphasis thus expressed is consistent with the expertise of one of the editors, Professor Johannes Weertman, who is attached to both the Materials and the Geology Departments at Northwestern University, Illinois (while the co-editor is professor of civil engineering at the same university). Up to now, most of the papers in MM have come from the United States, but there have been a number from elsewhere, notably from the geo-mechanical group in Paris headed by J.-P. Poirier and from M.S. Paterson's geophysics group in Australia.

The contributions tend to be strongly theoretical in slant, sometimes to the point of ultra-abstractness, but there are also a few experimental papers, some dealing with powders and porosity; and one intriguing work is concerned with the theory of the drying (seasoning) of wood, treated formally as a porous capillary body. Major theoretical categories include fracture mechanics and the theory of defect aggregates, dislocations in particular.

The new journal has two principal competitors (leaving out of consideration the various specialized fracture journals): the veteran *Journal of Mechanics and Physics of Solids*, now in its thirty-second volume and publishing about 500 pages per annum, a journal with distinct (British) Cambridge connections, and the newer *Res Mechanica* (subtitled *Structural Mechanics and Materials Science*), now on Vol. 11 and publishing around 1,000 pages each year. Both have a strong mathematical bias, like the new journal, and the coverage of all

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three overlaps considerably. Nevertheless, the geological leanings of MM, combined with the high standard of editing, suggest that it will survive even in the face of the excellent standards of its two immediate rivals.

The other journal, *Materials Chemistry and Physics* (MCP), is a re-titled version of *Materials Chemistry*, transferred to a new publisher from January 1983. In spite of the change of name, it still has a strong chemical bias and indeed the best of the many good papers are all chemical. The authors who fill the annual 1,200 pages or so at present are either Europeans (notably from Italy, France, Spain and eastern Europe) or from India. In this the journal differs from its established rivals, *Journal of Materials Science*, *JMS Letters*, *Materials Letters*, *Journal of Physics and Chemistry of Solids*, *Solid State Communications* (another letters journal) and *Materials Research Bulletin*. All of these, while international, have a strong American component. Further the new journal has a *de facto* emphasis on electrochemistry, especially photo-electrolysis, and also in general a more applied flavour than does *Journal of*

## MECHANICS OF MATERIALS

*Physics and Chemistry of Solids*. It has printed a number of valuable reviews, notably one on oxide electrode materials. Authors are invited to produce camera-ready copy once a typescript has been accepted, and many have availed themselves of this privilege if such it is; unfortunately some had no access to electric typewriters, which should surely be a precondition of acceptance of CRC, in courtesy to readers!

As, say, a journal of applied materials chemistry, I believe that MCP will find a permanent niche. But if it seeks to become a broad-spectrum materials journal (which it is not now), it risks becoming submerged by the competition. □

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## Fluent techniques

G.M. Lilley

### Experiments in Fluids: Experimental Methods and their Applications to Fluid Flow.

Editors W. Merzkirch and J.H. Whitelaw.

Springer-Verlag. 6/yr. DM 245, \$97.

THE study of theoretical and experimental fluid mechanics relies, as do most fields of science, on the publication in readily available form of good, independent research which has been carefully scrutinized and refereed before reaching print.

For the past 28 years the *Journal of Fluid Mechanics* has been the major forum in this field, and its record has been excellent. Subjects which are embraced by that publication are also among those proposed for this new English-language journal, *Experiments in Fluids* (EF). However, while there is merit in a new journal specifically dealing with experimental fluid mechanics, it would be regrettable if the *Journal of Fluid Mechanics* was thereby to reduce proportionally its number of papers on this important part of the subject; it would appear that there are enough acceptable papers to fill both journals.

The intention of the editors of EF is to publish research papers and technical notes relating to experimental methods and techniques, and to their application to the solution of problems in fluid mechanics. They specify that contributions to the journal should encompass a wide range of applications,

including aerodynamics, hydrodynamics, basic fluid dynamics, convective heat transfer, combustion, biological and geophysical flows and turbomachinery.

In the issues published to date there have been no editorial reviews or comments, or book reviews, but there seems to be no editorial policy excluding such features. Currently the publication time for articles appears to be very fast, with about a six-month gap between date of receipt and final publication. Authors receive 50 free off-prints of their published papers, a welcome bonus, but the journal's cost of \$97 per year for individuals seems rather expensive. The quality of production of articles is good, although there is nothing exceptional about the standard of illustrations.

The contributions published so far deal with both experimental techniques and detailed flow measurements, and certainly meet the requirements of the editors in respect of range of topics. In at least one paper attention is given to an uncertainty analysis of the measured data, but it cannot be overemphasized that such analysis should be given prominence in all papers published in a journal on experimental methods and measurements.

Although the need for a new journal within fluid mechanics can be questioned, there is probably a place for one specifically associated with experimental techniques and applications. On its early showing, EF appears to be a worthy newcomer in this field. □

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# Mantle enrichment processes

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*A review of Sr- and Nd-isotope and selected trace element data in both within-plate basalts and mantle xenoliths suggests that at least two enrichment processes are active within the upper mantle. One is consistent with the migration of small volume silicate melts with 'basanitic' trace element distribution patterns, whereas the other is characterized by relatively high  $\epsilon_{\text{Sr}}$ , Rb/Sr and K/Ti ratios, as observed in K-richertite-bearing periodotite xenoliths.*

THE Earth's mantle consists predominantly of Fe, Mg and Al silicates and it is probably fairly homogeneous with respect to major elements. However, mantle trace elements are distributed heterogeneously and these are particularly useful in attempting to describe different domains in the upper mantle and how they may have evolved. Trace elements are only present in very low concentrations and thus they rarely form minerals of their own. Many are termed incompatible because they do not sit readily in the crystal structure of common mantle minerals and so they concentrate in any small volume fluid or melt phase that may be present. Some incompatible elements are radioactive and potentially the changing isotope abundances of their daughter products allow evaluations of the age of individual mantle domains and of the timing of certain upper mantle processes. However, any interpretation of the observed variations in radiogenic isotopes should be allied to an understanding of the processes responsible for the distribution of the parent and daughter elements in the upper mantle. The composition of the mantle may be inferred from studies of basalts, and determined directly on rare samples of mantle rocks transported to the surface either tectonically or as xenoliths (fragments) in mantle-derived magmas. Such samples provide both mineralogical and chemical evidence for the changes that can result from processes that enrich and deplete trace elements in the upper mantle. This review considers the extent to which information on mantle trace-element enrichment processes from mantle xenoliths may be reconciled with some of the trace-element and radiogenic-isotope variations in recent basalts.

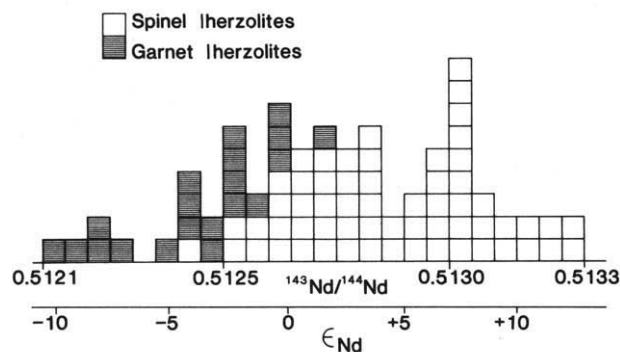
## Trace elements

The upper mantle is heterogeneous with respect to trace element ratios as well as abundances. Those portions that are enriched in trace elements relative to the mantle source of mid-ocean ridge basalts (MORB) or to best estimates of the primitive mantle testify to processes that scavenge and then concentrate these elements. Moreover, when trace-element enrichment results in changes in Rb/Sr, Sm/Nd and U/Pb, they will with time generate different Sr-, Nd- and Pb-isotope ratios. Yet, ironically, while the evidence for trace-element enriched areas in the upper mantle is generally accepted, there is widespread reluctance to believe that they may develop slightly unusual radiogenic isotope ratios.

The root of this reluctance arguably lies in one of the more spectacular applications of radiogenic isotopes, the models for the chemical evolution of the Earth<sup>1-3</sup>, for these models suggest that the chemical evolution of at least the outer portions of the Earth can be reasonably described in terms of a primitive mantle reservoir that differentiates to form the crust and a residual or depleted mantle. With the passage of time, and the radioactive decay of <sup>87</sup>Rb and <sup>147</sup>Sm, the crust evolves high <sup>87</sup>Sr/<sup>86</sup>Sr and low <sup>143</sup>Nd/<sup>144</sup>Nd ratios and the depleted (MORB-type) mantle has the complementary low <sup>87</sup>Sr/<sup>86</sup>Sr and high <sup>143</sup>Nd/<sup>144</sup>Nd.

Consequently, both have similar average model ages of 1,900–1,700 Myr. The problem is how to describe and interpret any compositions intermediate between the selected values for the crust and the depleted mantle. Within the confines of these models, the intermediate compositions can be described in terms of the chosen 'endmembers' of crust and depleted mantle. However, the success of the models has encouraged the two-component approach in other areas, in interpreting both the isotope ratios observed in individual magmatic provinces and the overall array of isotope data on mantle-derived rocks.

Figure 1 summarizes some of the Nd-isotope results available on fragments of the upper mantle brought to the surface as xenoliths in alkali basalts and kimberlites<sup>4-9</sup>. Most were determined on separated minerals that, unlike the whole rocks, appear not to have been contaminated with the host magma en route to the surface. The data range from high <sup>143</sup>Nd/<sup>144</sup>Nd ratios indistinguishable from those of MORB to low values often regarded as typical of crustal rocks. Thus not all mantle-derived rocks are depleted isotopically relative to the bulk Earth, and particularly in continental areas where old segments of mantle may be preserved within the continental lithosphere, it seems that mantle rocks can have comparable radiogenic isotope ratios to those in the overlying crust. For Nd, Sr and even Pb isotopes, caution is needed before arguing that isotope ratios which indicate trace-element-enriched source regions are necessarily derived from the crust. Crustal contamination may be better



**Fig. 1** Present day Nd-isotope composition of clinopyroxenes in spinel and garnet lherzolite inclusions from alkali basalts and kimberlites<sup>4-9</sup>.  $\epsilon_{\text{Nd}}(T)$  is the deviation from the value expected in a chondritic reservoir (CHUR) at time  $T$ , and is defined as

$$\epsilon_{\text{Nd}}(T) = 10^4 \left[ \frac{{}^{143}\text{Nd}/{}^{144}\text{Nd}_{\text{rock}}(T)}{{}^{143}\text{Nd}/{}^{144}\text{Nd}_{\text{CHUR}}(T)} - 1 \right]$$

where

$${}^{143}\text{Nd}/{}^{144}\text{Nd}_{\text{CHUR}}(T) = 0.51264 - 0.1967[(\exp \lambda_{\text{Sm}} T) - 1].$$

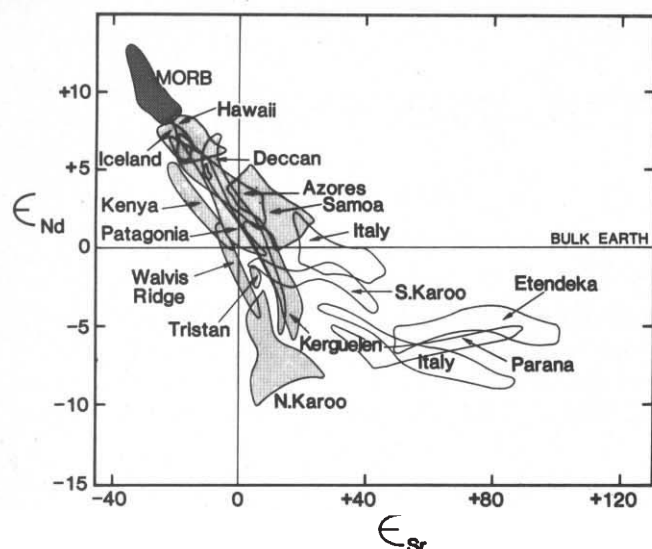


Fig. 2 Nd- and Sr-isotope compositions of selected Jurassic-Recent volcanic rocks<sup>12-28</sup>. The shading identifies MORB and two groups of within-plate basalts which tend to have different minor- and trace-element characteristics (see, for example, Figs 3, 4).

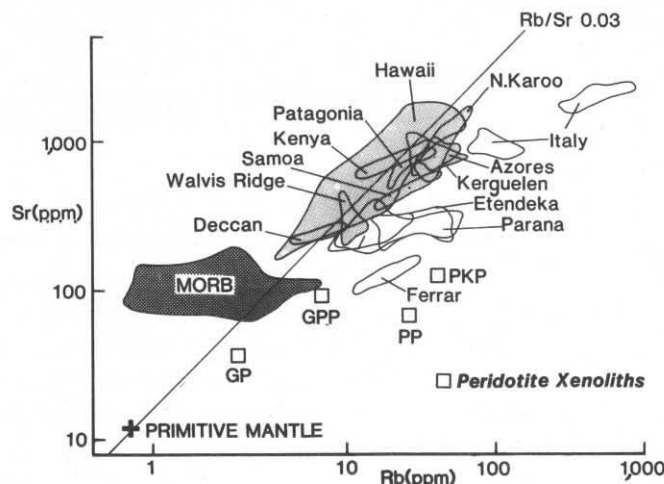


Fig. 3 Rb and Sr variations in selected MORB glasses, peridotite xenoliths and within-plate basalts<sup>5,12-26,35,75-84</sup>. For the peridotites and many basalt provinces average values have been plotted. Ferrar dolerites are included because of their high  $\epsilon_{Sr}$  values in primitive rocks<sup>42</sup>, even though  $\epsilon_{Nd}$  data are not available. Shading as in Fig. 2. GP, garnet peridotites; GPP, garnet phlogopite peridotites; PP, phlogopite peridotites; PKP, phlogopite K-rich peridotites<sup>5</sup>.

demonstrated by the changes in isotope composition that will result from any contamination process, than by appealing to the discriminating powers of specific radiogenic isotope ratios.

The counter argument to such evidence for a mantle relatively enriched in radiogenic isotopes is that while xenoliths in kimberlites are interesting in their own right, they might come from anomalous segments of mantle and so might not be relevant to the study of basalts and their mantle source regions. The evidence from xenoliths need not, therefore, deflect attempts to model the isotope ratios of within-plate basalts in terms of crust and depleted mantle. Moreover, because most xenoliths from kimberlites are too impoverished in those major elements that are enriched in basalt, and are therefore incapable of producing much basaltic melt<sup>10</sup>, it is not an argument that can be lightly dismissed.

One way of assessing the significance of mantle enrichment processes is to compare not simply the isotope ratios in basalts and xenoliths, but also variations in minor and trace elements, and hence the coherence between isotope and trace element compositions<sup>11</sup>. This review therefore focuses on certain aspects of the minor- and trace-element variations in mantle xenoliths and basalts which, in conjunction with the Nd- and Sr-isotope data, offer some constraints on the nature of mantle enrichment processes. The discussion is deliberately restricted to Nd and Sr isotopes because their distribution and that of their parents Sm and Rb may be more readily related to that of other commonly analysed trace elements than can, for example, the rare gases or even U and Pb.

## Nd and Sr isotopes

Detailed studies have been carried out on a wide range of both oceanic and continental volcanic rocks and some of the results are summarized in Fig. 2 (refs 12-28). Data have been omitted both for clarity, where they exhibit similar trends to those already plotted, and where a reasonable case exists for contamination with continental material as mantle-derived magmas migrate up through the crust (for example, the south Lebombo basalts of the Karoo<sup>24</sup>, the lower lavas of the Mahabaleshwar section of the Deccan Traps<sup>20,27</sup> and the British Tertiary<sup>29</sup>). Overall, the inverse correlation of  $\epsilon_{Nd}$  and  $\epsilon_{Sr}$  defines a broad swathe of results through a point representing the estimated composition of the undifferentiated bulk Earth<sup>28</sup>. Most basalts have positive  $\epsilon_{Nd}$  and negative  $\epsilon_{Sr}$  (low  $^{87}Sr/^{86}Sr$ ) values and were, therefore, derived from source regions which had low Rb/Sr and Nd/Sm for much of their history; that is, they were relatively depleted

in the more incompatible elements such as Rb and the light rare-earth elements (LREEs). Conversely, rocks with negative  $\epsilon_{Nd}$  and positive  $\epsilon_{Sr}$  contain at least a contribution from sources that had high Nd/Sm and Rb/Sr ratios, and hence by implication were relatively enriched in incompatible elements, for long periods of time. Many crustal rocks have similar  $\epsilon_{Nd}$  and  $\epsilon_{Sr}$  values, but then so too do some peridotite mantle xenoliths and the diopsides separated from them (refs 4, 5, 30 and Fig. 1).

The issue is the extent to which the broad trend of the Nd- and Sr-isotope data reflects mantle processes operating on the global scale, including the recycling of continental material. The models of Earth evolution rely heavily on the complementary nature of the crustal and depleted (MORB-type) mantle reservoirs, and the shape of the data array is broadly consistent with two-component mixing. Thus, there has been much speculation that this array is due primarily to mixing between continental and depleted mantle material, with the former being introduced either in subducted ocean crust<sup>18,30</sup> or as portions of the continental lithosphere re-assimilated back into the convecting upper mantle<sup>31,32</sup> or by straightforward contamination of mantle-derived magmas en route to the surface in continental areas<sup>33</sup>. However, recent basalts also exhibit a curved negative correlation between Sm/Nd and Rb/Sr (ref. 17). This is consistent with the available information on the distribution of those elements between coexisting mantle phases<sup>34</sup>, and it implies that the  $\epsilon_{Nd}-\epsilon_{Sr}$  array might equally reflect the fractionation of Sm/Nd and Rb/Sr in a whole series of localized enrichment and depletion events in the upper mantle.

Figure 2 shows the data on intraplate basalts, subdivided on the basis of  $\epsilon_{Nd}$  and  $\epsilon_{Sr}$ , and the minor and trace element variations illustrated in Figs 3 and 4. One group largely plots on or to the left of the steep portion of the array, with the possible exception of those from Samoa and perhaps the Azores which are discussed below, whereas the samples in the other group are displaced to relatively high  $\epsilon_{Sr}$  values. Moreover, while rocks in the first group occur in both oceanic and continental areas, those with relatively high  $\epsilon_{Sr}$  seem to be restricted to the continents. This observation suggests that mantle source regions with similar trace element histories are sampled in both environments, and that the high  $\epsilon_{Sr}$  values are due to contamination with continental crust and/or to a different trace-element enrichment process apparently confined to the sub-continental mantle.

Trace-element studies have demonstrated that some basalts have high concentrations of incompatible elements, including



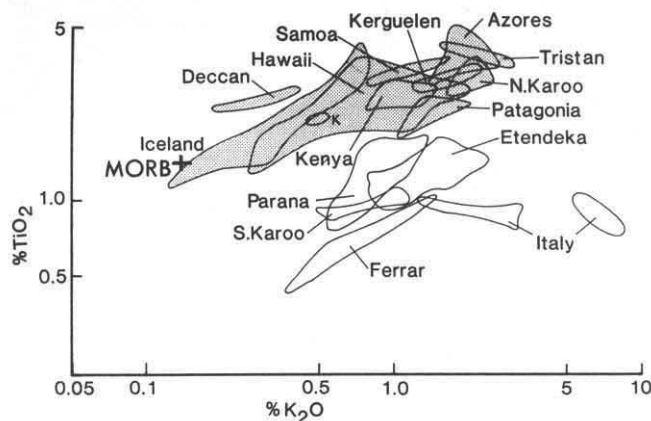


Fig. 4  $\text{TiO}_2$  and  $\text{K}_2\text{O}$  variations in the basic volcanic rock suites for which  $\epsilon_{\text{Nd}}$  and  $\epsilon_{\text{Sr}}$  data are plotted in Fig. 2. The exceptions are the Ferrar dolerites which are included because of their high  $\epsilon_{\text{Sr}}$  values, although  $\epsilon_{\text{Nd}}$  data have not yet been published. Shading as in Fig. 2. K, small subset of Kerguelen data. Typical MORB from ref. 40.

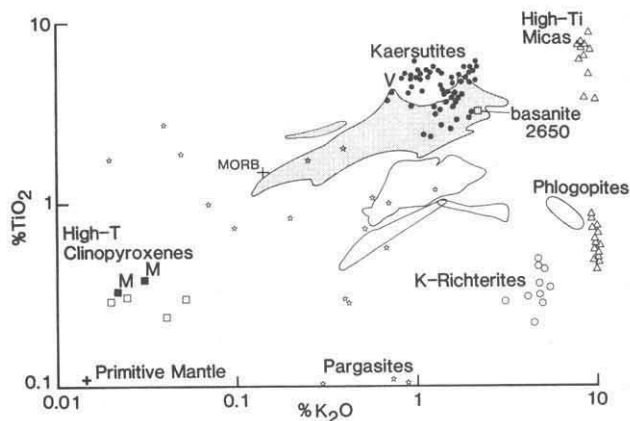


Fig. 5  $\text{TiO}_2$  and  $\text{K}_2\text{O}$  contents of micas, amphiboles and high-temperature clinopyroxenes<sup>45-50,52-61,67-71</sup> compared with trends identified in basalts in Fig. 4. The high Ti-micas, kaersutites and pargasites are from inclusions in alkali basalts, whereas the phlogopites, K-richterites and clinopyroxenes are from inclusions in kimberlite. V, vein sample; M, megacryst from kimberlite. Open squares, diopsides from high-temperature peridotites.

the LREEs, accompanied by comparatively low or unfractionated Rb/Sr ratios, while in others high incompatible element contents are accompanied by high Rb/Sr ratios<sup>17,21,23,24,35</sup>. These differences seem to reflect different trace-element enrichment processes in the upper mantle, and with time they will generate low  $\epsilon_{\text{Sr}}$ , low  $\epsilon_{\text{Nd}}$  and high  $\epsilon_{\text{Sr}}$ , low  $\epsilon_{\text{Nd}}$  trends respectively on the Nd-Sr isotope diagram.

The case for the existence of different trace-element enrichment processes is supported by studies on garnet- and amphibole-bearing peridotite xenoliths from kimberlite pipes in southern Africa<sup>4,5,36</sup>, and by studies on apatite pyroxenite inclusions from south-east Australia<sup>37</sup>. Most cold, coarse peridotites from southern Africa are enriched in LREEs, and hence have low Sm/Nd ratios, but those which contain garnet  $\pm$  phlogopite are characterized by low Rb/Sr, in contrast to those that are garnet-free and contain phlogopite  $\pm$  K-richterite and have high Rb/Sr ratios (see average values on Fig. 3). With time the low Sm/Nd, low Rb/Sr rocks have evolved on or to the left of the  $\epsilon_{\text{Nd}}-\epsilon_{\text{Sr}}$  array, and the high Rb/Sr, low Sm/Nd samples have generated relatively high  $\epsilon_{\text{Sr}}$ , low  $\epsilon_{\text{Nd}}$  values comparable with those in the Italian and South Karoo basalts in Fig. 2 (refs 11, 23). Furthermore, if mantle-enrichment processes are primarily responsible for such coherent variations in Rb/Sr and Sm/Nd (and hence  $\epsilon_{\text{Sr}}$  and  $\epsilon_{\text{Nd}}$ ), they should also result in other minor- and trace-element differences that might, for example, be more sensitive to the nature of those enrichment processes.

## Rb, Sr and $\text{TiO}_2$ , $\text{K}_2\text{O}$

There have been surprisingly few attempts to analyse minor- and trace-element variations in mantle-derived rocks in the context of mantle-enrichment processes, and there is only room here for some preliminary speculations. It has been demonstrated<sup>5,11,24</sup> that Rb/Ba ratios were remarkably similar in both garnet and garnet-phlogopite lherzolite xenoliths from Kimberley and in many Karoo basalts, even though average Ba contents ranged from 19 p.p.m. in xenoliths up to 920 p.p.m. in northern Karoo lavas. All these rocks plotted on or to the left of the  $\epsilon_{\text{Nd}}-\epsilon_{\text{Sr}}$  array, and any group of rocks that had high Rb/Ba ratios also had relatively high  $\epsilon_{\text{Sr}}$  values (for example, the Etendeka<sup>24</sup> in Fig. 2). However, there are fewer Rb/Ba than Rb/Sr data available in the literature, and since variations in Rb/Sr may also be related more directly to those in  $^{87}\text{Sr}/^{86}\text{Sr}$ , Rb and Sr results from some basalts and mantle xenoliths are outlined in Fig. 3 to investigate this line of argument further.

Where possible, the analyses used in compiling Fig. 3 are on the same rocks as those in Fig. 2, although for many provinces, average Rb and Sr values have been plotted. This is partly because in some instances only average values are available, and partly for clarity because it minimizes the scatter. The averages are based on local criteria such as magma type, stratigraphical unit and radiometric age. What is striking is that even though Rb/Sr ratios are highly susceptible to low-pressure effects such as plagioclase fractionation, there are still strong links between Rb/Sr and  $\epsilon_{\text{Sr}}$  (refs 38, 39). The basalts with high Rb/Sr have relatively high  $\epsilon_{\text{Sr}}$ , and those with comparatively unfractionated Rb/Sr ratios plot on or to the left of the steep portion of the  $\epsilon_{\text{Nd}}-\epsilon_{\text{Sr}}$  array.

Such consistency between isotope- and trace-element ratios is reassuring, but it is difficult to evaluate by studies of mantle xenoliths because of practical problems in obtaining representative, or even reliable, trace-element data from many mantle rocks. However, several studies have reported high quality microprobe analyses on mantle minerals so that if the inferred styles of trace-element enrichment could be recognized in at least minor element variations, it should be possible to evaluate them by comparison with the minor-element variations in mantle minerals.

The minor elements selected were Ti and K and the results were treated in the same way as the Rb and Sr data. Most basalts from both oceanic and continental areas plot on a broad array within which Ti/K decreases from  $\sim 7$  in typical MORB<sup>40</sup> to  $\sim 1.4$  in incompatible element-enriched basalts on oceanic islands such as Sao Miguel in the Azores and Tristan da Cunha (Fig. 4). Significantly, however, those basalts which have relatively high  $\epsilon_{\text{Sr}}$  (Fig. 2) appear to be characterized by relatively low Ti contents, and hence low Ti/K ratios ( $\sim 1-0.1$ ). Once again high  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios consistently occur in rocks which have distinctive minor- and trace-element features. Crustal contamination will tend to reduce Ti/K, but significant amounts of contamination are required if that is to be the cause of the observed low Ti/K ratios. For example, to generate a basalt with 1%  $\text{TiO}_2$  and 1%  $\text{K}_2\text{O}$  from typical MORB<sup>40</sup> implies 30% contamination, even in the most advantageous conditions of a  $\text{TiO}_2$ -free contaminant (whereupon the contaminant would have  $\sim 2.5\%$   $\text{K}_2\text{O}$ ), and the amount of contamination is not reduced by invoking assimilation with fractional crystallization<sup>41</sup> and/or a contaminant with higher  $\text{K}_2\text{O}$ . Yet both the high Ti and low Ti trends include primitive basalts with so-called 'mantle'  $\delta^{18}\text{O}$  values of  $6.0 \pm 0.5\%$  (refs 42, 43, and R. S. Harmon, personal

communication), suggesting that it is most improbable that the difference between the two trends is due to crustal contamination.

Further evidence for attributing different Ti–K trends to mantle processes comes from the variation of Ti contents, in particular in mantle phases. The textural relations of, for example, phlogopite in mantle xenoliths are extremely complex, and while many authors have identified ‘primary’ and ‘secondary’ phlogopite mica<sup>44–50</sup>, it is often unclear whether the latter reflect mantle metasomatic processes or interaction with the host kimberlite. In general, secondary micas have higher and more variable Ti contents and in this discussion we have assumed that they reflect kimberlite interaction, and so have not considered them further. Primary phlogopites have  $\leq 1\%$  TiO<sub>2</sub> (ref. 46) consistent with the low TiO<sub>2</sub> contents of the amphibole with which they are most often associated K-richterite (see Fig. 5) and the low Ti/K whole rock ratios of the phlogopite–richterite peridotite suite<sup>48</sup>. The identification of Ti-rich accessory phases in association with richterite<sup>51</sup> indicates that Ti is also mobilized in this process, even though the overall character is one of alkali enrichment. Moreover, since these metasomatized xenoliths also exhibit relatively high Rb/Ba, Rb/Sr and  $\epsilon_{\text{Sr}}$  values<sup>5</sup>, they have both minor- and trace-element and Nd–Sr isotope features remarkably similar to those of the low Ti/K, high  $^{87}\text{Sr}/^{86}\text{Sr}$  continental basalts (Figs 2, 4).

Spinel peridotites also exhibit isotope- and trace-element features consistent with trace-element enrichment after some previous major-element depletion episode<sup>49,50</sup>. However, unlike garnet peridotites, spinel peridotites occur predominantly in alkali basalt provinces, and ‘metasomatism’ tends to be associated with multiple intrusions of alkali-rich or ‘basaltic’ silicate magma<sup>52–61</sup>. Composite xenoliths preserve a record of pyroxenite veins cross-cutting peridotite, and the common amphibole is kaersutite which can occur in veins, poikilitically within the host lherzolite or as megacrysts in alkali-rich basalts. Phlogopite is rare, and when present it is usually Ti-rich, so that both the amphibole and mica commonly associated with trace-element enrichment in the spinel lherzolite facies have the relatively high Ti/K ratios characteristic of most oceanic and continental within-plate basalts. Moreover, both the basalts on the high Ti/K trend and clinopyroxenes and kaersutites from spinel lherzolites tend to have low  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios,  $<0.7055$  (ref. 50).

The amphibole pargasite also occurs as megacrysts and in spinel lherzolites, and yet it is characterized by highly variable K and Ti contents (Fig. 5) and locally it is associated with low-Ti phlogopite<sup>62</sup>. However, the scatter of K and Ti data indicates that pargasite has no simple relationship with the processes responsible for the coherent trends in the basalts. It has been argued, for example, that pargasite may form during wall rock alteration around magma conduits<sup>61</sup>.

## Magma migration or metasomatism?

The descriptions of trace-element enrichment processes inferred from mantle xenoliths vary significantly from spinel- to garnet-bearing suites. The former emphasize the role of associated magmatism; veins and megacrysts testify to the transfer of material in silicate melts, and chemical metasomatism of the country rocks may be largely restricted to the contacts with veins and conduits. In contrast, discussions of xenoliths from kimberlites, particularly from the Kimberley area where most work has been done on K-richterite bearing rocks<sup>5,46</sup>, often appeal to ‘pervasive metasomatism and the infiltration of H<sub>2</sub>O-rich fluids’, and comparatively few vein textures have been reported. Thus, it is very tempting to attribute the low Ti/K, high Rb/Ba, Rb/Sr and subsequently high  $\epsilon_{\text{Sr}}$  style of enrichment recognized in both basalts and mantle xenoliths in this form of mantle metasomatism, to the infiltration of H<sub>2</sub>O- and alkali-rich fluids. Such fluids probably reflect different processes in different areas, but there is increasing evidence from the Italian volcanics (see Figs 2–4) that, at least locally, this style of trace-element enrichment can be related to subduction<sup>62,63</sup>. The more common high Ti/K

style of enrichment occurs in both oceanic and continental areas, and the evidence from spinel lherzolites and megacrysts indicates that it reflects the migration of small volumes of silicate melt. It concentrates a spectrum of trace elements from Rb, K, Ba, through Nb, Ta and the LREEs, to Zr and Ti, with the result that within-plate basalts may be characterized on discriminant diagrams intended to distinguish basalts erupted in different tectonic settings<sup>64</sup>, and many of the more incompatible element-enriched basalts on the high Ti trend have trace-element-normalized patterns similar to those of basanites. LREE abundances increase, and so Sm/Nd ratios are low, but there is relatively little increase in Rb/Sr and the basalts and xenoliths consistently plot on, or to the left of, the steep part of the  $\epsilon_{\text{Nd}}-\epsilon_{\text{Sr}}$  array.

These features appear to be characteristic of enrichment processes in the convecting upper mantle and they are also observed in discrete nodules (or megacrysts) and high-temperature deformed peridotites from kimberlite pipes<sup>65,66</sup>. Clinopyroxene megacrysts and separates from high-temperature peridotites have both low  $^{87}\text{Sr}/^{86}\text{Sr} = 0.7024-0.7036$  (refs 67–69) and high Ti/K ratios<sup>70,71</sup> comparable with those of the high Ti/K basalt trend (Fig. 4). Thus the isotope- and trace-element compositions, and the pressure–temperature (P–T) conditions of equilibration, of the high-temperature peridotite are predominantly asthenospheric in character; as are the megacrysts which appear to have crystallized from high-temperature Ti-rich magmas derived from the convecting upper mantle. Harte<sup>66</sup> has recently developed this argument to show that using partition coefficients for basaltic magmas the estimated trace-element composition of magma in equilibration with a clinopyroxene megacryst from kimberlite is very similar to that of basanite 2650 from Frey *et al.*<sup>34</sup> (see Fig. 5), which is believed to be a good example of a small-volume mantle melt.

## Discussion

This preliminary review of minor- and trace-element and Nd–Sr isotope data on within-plate basalts and on xenoliths containing hydrous mantle phases suggests that at least two enrichment processes are active in the upper mantle. In reality there is probably a continuum of enrichment processes. Volcanic rocks with  $\epsilon_{\text{Nd}}-\epsilon_{\text{Sr}}$  values on, or to the left of, the mantle array have trace-element compositions consistent with the migration of small volumes of ‘basaltic’ melts within their upper mantle source regions. This seems to be the dominant enrichment process in both oceanic and continental areas and it can be observed directly in the composite spinel lherzolite xenoliths which are a feature of many young alkali basalt provinces. In addition, basaltic high-Ti, low  $^{87}\text{Sr}/^{86}\text{Sr}$  magmas have been in equilibrium with the megacryst suite in kimberlites and responsible for the Fe–Ti enrichment in high-temperature deformed peridotites<sup>65</sup>. In contrast, basalts from some provinces are characterized by relatively high  $\epsilon_{\text{Sr}}$ , Rb/Ba, Rb/Sr and K/Ti—as are metasomatized phlogopite + K-richterite-bearing peridotite xenoliths. These represent a different style of mantle enrichment which is apparently confined to continental areas. It probably results from more than one process, but there is evidence from the Italian volcanics, for example, that, at least locally, it can be related to the release of H<sub>2</sub>O- and alkali-rich fluids above a subduction zone<sup>62,63</sup>.

There has been some debate over the timing of mantle-enrichment events and their relation to volcanism<sup>72</sup>. In many areas, trace-element enrichment seems to have been closely associated with, and may locally have been responsible for, magma genesis<sup>9,17,73</sup>. Interestingly, however, such magmatic provinces all seem to lie on the high Ti/K trend and it has been suggested that this enrichment reflects the migration of small-volume partial melts. The low Ti/K style of enrichment seems to have no such simple relationship to magma genesis, although in the case of the K-richterite metasomatism in xenoliths from southern Africa, it takes place soon after the widespread Karoo magmatism (ref. 11 and refs therein).

Note that none of the figures used here are intended to be discriminant diagrams, particularly in assessing the effects of



crustal contamination. The analyses plotted in Figs 2–4 are from suites in which at least the primitive rocks do not seem to have been affected significantly by crustal contamination. So, although it has been argued that the observed trends are due to mantle-enrichment processes, some of the effects, such as high  $\epsilon_{\text{Sr}}$ , can also result from crustal contamination. The case for crustal contamination must be reviewed separately for each area, and with the possible exception of high  $\delta^{18}\text{O}$  values, it leaves few unambiguous fingerprints.

Finally, there is the question of the extent to which these postulated trace-element-enrichment processes are responsible for the observed range in  $\epsilon_{\text{Nd}}$  and  $\epsilon_{\text{Sr}}$  in recent basalts, or whether the latter primarily reflect an additional component such as subducted crust<sup>18,30</sup> or re-assimilated continental lithosphere<sup>32</sup>. The coherence between the minor- and trace-element and the isotope trends implies that they are related, and the observed range in Rb/Sr (Fig. 3) and Sm/Nd (which decreases as Rb and Sr contents increase, Fig. 3) appears to be sufficient to generate the variations in  $\epsilon_{\text{Nd}}$  and  $\epsilon_{\text{Sr}}$ . This suggests that an additional (continental) component may not be necessary to explain the trends in  $\epsilon_{\text{Nd}}-\epsilon_{\text{Sr}}$ , except perhaps in areas where inconsistencies exist between the isotope and trace-element data. Such a case may be made for the Azores and Samoa<sup>18</sup> rocks because they have slightly high  $\epsilon_{\text{Sr}}$  (Fig. 2) and yet still plot on the high Ti/K trend (Fig. 4), but whether that inconsistency is sufficient to require a recycled component to explain the isotope results remains conjectural. It has also been suggested that the

$\epsilon_{\text{Nd}}$ ,  $\epsilon_{\text{Sr}}$  values of ocean island basalts may primarily reflect the recycling of high  $\epsilon_{\text{Sr}}$  material from the continental lithosphere<sup>32</sup>. However, such material seems to be characterized by low Ti/K ratios so that it could not control the volumetrically dominant high Ti/K trend in Figs 4, 5—and that would imply that the links between the isotope and minor-element trends identified here are coincidental.

Continental material appears to be recycled into the upper mantle by subduction<sup>18,30</sup>, and it may well have influenced the actual  $\epsilon_{\text{Nd}}$  and  $\epsilon_{\text{Sr}}$  values estimated for the average upper mantle<sup>74</sup>. However, the systematic minor- and trace-element differences in basalts and mantle xenoliths which exhibit different Nd- and Sr-isotope trends suggests that the relative fractionation of  $\epsilon_{\text{Nd}}$  and  $\epsilon_{\text{Sr}}$  is closely related to the postulated trace-element enrichment processes. The dominant process results in high Ti/K, low Sm/Nd and little fractionation of Rb/Sr, consistent with the migration of small-volume partial melts within the convecting upper mantle. Such melts can be derived from variously depleted and more primitive mantle reservoirs and convection will probably ensure that the enriched portions of mantle are comparatively short-lived, being continually created and destroyed.

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# Casimir effect for massive photons

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*The suggestion that the force between two perfectly conducting plates in quantum electrodynamics depends critically on whether the photon mass is precisely zero or not is false. The solution to this problem involves the discovery of a new class of classical solutions for the electromagnetic field between conducting plates, when the photon has non-zero mass.*

THE inverse-power-law forces that act between macroscopic bodies as a consequence of the van der Waals forces between their constituents are not unexpected. They are understood as ordinary electromagnetic forces between charge and current fluctuations at the molecular level. One might then expect a similar interaction between (idealized) perfectly conducting bodies, obtainable from the theory for ordinary conductors and dielectrics by some appropriate limiting procedure.

In contrast to such explanations of these interactions, which focus on charges and currents, the forces between perfect conductors can be understood very simply and directly from an alternative point of view which focuses on the electromagnetic field, and ascribes the forces to changes in the quantum zero-point energy of the field normal modes when the conductors are introduced. The conductors affect the normal modes through the boundary conditions which the fields must satisfy on the conducting surfaces. Accordingly the forces depend only on Planck's constant  $\hbar$  (characteristic of quantum mechanics), the speed of light  $c$  (characteristic of Maxwell's electromagnetic field equations), and on purely geometrical factors (characteristic of the spatial configuration of the conductors). The archetype of all such phenomena is the Casimir effect<sup>1</sup>, the attractive force  $F$  (per unit area) between two perfectly conducting slabs separated by a distance  $2L$

$$F = -\frac{\pi^2}{240} \frac{\hbar c}{(2L)^4} = -\frac{\partial U}{\partial (2L)}$$

$$U = -\frac{\pi^2}{720} \frac{\hbar c}{(2L)^3}$$

The connection between this approach and the more conventional one has been much discussed by, for instance, Lifshitz and Pitaevskii<sup>2</sup> and Tabor<sup>3</sup>. Here we shall be concerned only with the Casimir approach.

Davies and Unwin<sup>4</sup> considered the problem of the Casimir effect between perfectly conducting plates separated by a gap of length  $2L$ , assuming that the photon has a small non-zero mass  $m$ . This implies that longitudinal modes of the electromagnetic field are present in the gap in addition to the usual transverse modes given by Maxwell's theory. Davies and Unwin conclude that either: (1) the Casimir effect in this case is 3/2 times as large as the effect for  $m=0$  (because an additional longitudinal mode is present as well as the two transverse modes for each allowed frequency), or (2) the quantum-mechanical electromagnetic energy-momentum tensor diverges near a conducting plate, leading to a discontinuous change in the surface energy of the plate as compared with  $m=0$ . Case (1) corresponds to an assumption of perfect reflection of the longitudinal modes; case (2) corresponds to the assumption of perfect transmission.

These results, if true, would be surprising. Stueckelberg<sup>5</sup> first investigated quantum electrodynamics (QED) with a massive photon and concluded that amplitudes describing longitudinal

modes tended continuously to zero as  $m \rightarrow 0$ , provided that the electromagnetic current were conserved. This result was extended by Goldhaber and Nieto<sup>6</sup> who state that "in the massive photon case the fields and currents of a system are changed only by order  $(mD)^2$ " where  $D$  is the appropriate linear dimension of the system and we now use units  $\hbar = c = 1$ . It is, therefore, difficult to see how Davies and Unwin's discontinuous results can materialize in a macroscopic system when the situation is continuous on the microscopic level.

On the other hand, if a 'graviton' or Yang-Mills 'photon' is given a small mass, then the resultant theory in the zero mass limit is not the original zero mass theory<sup>7-9</sup>. These matters are of current interest in particle physics because the presence of a small photon mass is not allowed in a non-Abelian grand unified gauge theory such as SU(5) containing electrodynamics but is allowed in a theory which is not unified such as SU(3)  $\times$  SU(2)  $\times$  U(1) (ref. 10).

Classical electrodynamics, with finite photon mass, contains a new characteristic length scale, the Compton wavelength  $\lambda = m^{-1}$  of the photon. Generally, a small non-zero photon mass will lead to new physical effects over a length scale  $\lambda$ . In particular, the effective range of electromagnetic interactions will be exponentially damped by  $e^{-r/\lambda}$  and even for small values of  $m$  this leads to important effects over astronomical length scales. Because of this, the strongest indirect limits on  $m$  come from astrophysics<sup>11</sup>. They all arise in the same way: by considering astronomical structures in which magnetic fields are known to have a key role in maintaining equilibrium under the assumption that  $m$  is zero. The hypothesis that  $m \neq 0$  will then destroy the conditions for equilibria over length scales  $r > \lambda$ , and hence one obtains an upper limit on  $m$ . Considerations of the Galactic magnetic field and magneto-acoustic propagation in the Crab Nebula<sup>12-14</sup> lead, in this way, to upper limits on  $m$  of order  $10^{-20}$  eV. Magnetized interstellar gas clouds and interstellar gas in the disk of the Milky Way seem to require supporting magnetic field structure and hence<sup>12,13</sup> produce upper limits of about  $10^{-25}$  eV. The strongest published limit on  $m$  results from an application of this argument<sup>15,16</sup> to magnetically supported structures in the Small Magellanic Cloud which are claimed to exist over length scales up to about 3 kpc. This leads to an upper limit of  $m \leq 10^{-27}$  eV but should be examined in more detail.

Astronomical interest in the question of photon mass has been revived by the suggestion of Georgi *et al.*<sup>17</sup> that photon mass permits photon oscillations which could leave observable traces in the microwave background radiation in the centimetre range. Unfortunately, a high value of  $m \sim 5 \times 10^{-18}$  eV seems to be required for an observable sinusoidal distortion of the black-body spectrum of the radiation.

Another area in which photon mass may have important consequences has been discussed by Fargion<sup>18</sup> who points out that the characteristic mass of gravitational Bose condensation in a massive photon gas is  $\sim 7.5 \times 10^8 (m/10^{-19} \text{ eV})^{-1} M_\odot$ . This mass scale is similar to that of quasars and the nuclei of giant



galaxies. It is also a mass scale that would lead to an acceptable picture of gravitational clustering, if it were the scale above which density inhomogeneities could survive damping in the early Universe<sup>19</sup>.

This article shows that Stueckelberg *et al.* are right as far as the Casimir effect between two perfectly conducting plates is concerned: there are no discontinuities in photon mass  $m$  as  $m \rightarrow 0$ . Neither is there any discontinuity in surface energy. Davies and Unwin obtain incorrect results because they postulate incorrect boundary conditions: they have no freedom to choose boundary conditions because those are determined in advance by the Proca equations describing the classical electrodynamics of a massive photon. The solutions of the Proca equations are unique just as they are in Maxwell's theory.

We, therefore, outline here the solution to the Casimir effect problem assuming a photon of mass  $m$ . The details of the calculation will be given elsewhere<sup>20</sup> but we think that the solution involves material of general interest especially in view of the implications for particle physics and astronomy of the existence of a massive photon. The full normal mode analysis of the Proca equations is rather delicate, even in the apparently simple case of slab geometry, and the relations between certain Proca modes and the maxwellian modes are less obvious than they might seem. A systematic treatment is given in ref. 20; the statements that follow are a somewhat simplified version which is not misleading as far as the calculation of the Casimir effect is concerned. We will show that: (1) there are two independent solutions of the Proca equations for the electromagnetic field between two perfectly conducting plates which correspond to the two independent and transverse solutions of Maxwell's theory. The eigenvalue equation which determines the allowed discrete values of the wave number  $p$  perpendicular to the plates is unchanged; (2) there is a new class of solutions with a continuous spectrum in  $p$  for the Proca equations, which does not exist for Maxwell's equations; (3) the Casimir force receives contributions from both solutions (1) and (2) for non-zero  $m$ . We calculate these and show that the  $m \rightarrow 0$  limit is smooth.

We also demonstrate that the solutions (in class 2) of the Proca equations, which have not been written down before as far as we are aware, are mathematically identical to the one-dimensional square well problem of Schroedinger quantum mechanics. We do not calculate the surface energy here, but we quote the result which is smooth as  $m \rightarrow 0$ .

## Proca equations

Potentials  $\mathbf{A}$ ,  $\phi$  are introduced in the Proca equations<sup>11</sup> as in Maxwell's theory satisfying the Lorentz condition

$$\nabla \cdot \mathbf{A} + \frac{\partial \phi}{\partial t} = 0 \quad (1)$$

and the field strengths are given by

$$\mathbf{E} = -\frac{\partial \mathbf{A}}{\partial t} - \nabla \phi, \quad \mathbf{B} = \nabla \times \mathbf{A} \quad (2)$$

The energy density  $T^{00}$  of the electromagnetic field is given by

$$T^{00} = \{(\mathbf{E}^2 + \mathbf{B}^2) + m^2(\mathbf{A}^2 + \phi^2)\}/8\pi \quad (3)$$

so that  $\mathbf{A}$  and  $\phi$  are now physical fields which cannot be transformed away. In the presence of sources  $\mathbf{j}$ ,  $\rho$  satisfying

$$\nabla \cdot \mathbf{j} + \frac{\partial \rho}{\partial t} = 0 \quad (4)$$

we have

$$\nabla \cdot \mathbf{E} = 4\pi\rho - m^2\phi \quad (5a)$$

$$\nabla \times \mathbf{B} = 4\pi\mathbf{j} - m^2\mathbf{A} + \frac{\partial \mathbf{E}}{\partial t} \quad (5b)$$

Following Kroll<sup>21</sup>, we can now look for monochromatic plane

wave solutions of these equations of positive frequency  $\omega$ . We must have

$$\nabla \cdot \mathbf{A} - i\omega\phi = 0 \quad (6)$$

$$\nabla^2 \mathbf{A} + \omega^2 \mathbf{A} - m^2 \mathbf{A} = -4\pi\mathbf{j} \quad (7a)$$

$$\nabla^2 \phi + \omega^2 \phi - m^2 \phi = -4\pi\rho \quad (7b)$$

For  $\omega < m$ ,  $q^2 = m^2 - \omega^2$ , the solutions of equations (7) can be written as

$$(\mathbf{A}(\mathbf{r}), \phi(\mathbf{r})) = \int \frac{(\mathbf{j}(\mathbf{r}'), \rho(\mathbf{r}')) e^{-q|\mathbf{r}-\mathbf{r}'|}}{|\mathbf{r}-\mathbf{r}'|} d\mathbf{r}' \quad (8)$$

in terms of sources  $\rho$ ,  $\mathbf{j}$ . It follows that  $\phi$  and  $\mathbf{A}$  are uniquely determined by the sources and are everywhere continuous provided  $\mathbf{j}$  and  $\phi$  are not too singular. In particular, introducing the conductivity  $\sigma$  as usual through  $\mathbf{j} = \sigma\mathbf{E}$ , the case of a perfect conductor  $\sigma = \infty$  is defined simply by taking  $\mathbf{E} = 0$  in the conductor. In this case<sup>21</sup>,  $\mathbf{A}$  and  $\phi$  are still continuous across the boundary of the conductor although  $\mathbf{E}$  and  $\mathbf{B}$  may be discontinuous.

It is easy to extend the solutions (8) to  $\omega > m$  by analytical continuation. We can now investigate the Proca solutions for the fields between two perfectly conducting plane parallel plates.

## Maxwellian solutions

We begin with the treatment of the maxwellian case following ref. 22. Take one conducting plate to be at  $z = 0$ . Then two independent solutions for  $z > 0$  satisfying  $\nabla \cdot \mathbf{A} = 0$ , and  $A_x = A_y = 0$  on the plate are

s-mode:

$$\mathbf{A} = e^{-i\omega t} e^{i\mathbf{k} \cdot \boldsymbol{\rho}} \sin pz (\mathbf{k} \times \hat{z})/\omega \quad (9)$$

where  $\mathbf{k}$  is a wave vector parallel to the plates,  $\boldsymbol{\rho} = (x, y)$ ,  $\hat{z}$  is the unit vector in the  $z$ -direction (perpendicular to the plates) and  $\omega^2 = \mathbf{k}^2 + p^2$ .

p-mode:

$$\mathbf{A} = e^{-i\omega t} e^{i\mathbf{k} \cdot \boldsymbol{\rho}} (i\mathbf{k} p \sin pz - \hat{z} \mathbf{k}^2 \cos pz)/\omega^2 \quad (10)$$

Now consider a conducting plate at  $z = 2L$ . Taking  $A_x = A_y = 0$  at  $z = 2L$  gives the eigenvalue equation in both cases

$$\sin 2pL = 0 \quad (11)$$

or

$$p_n = n\pi/2L, \quad \text{integral } n \quad (12)$$

Thus the allowed frequencies for any  $\mathbf{k}$  are

$$\omega_n^2 = p_n^2 + \mathbf{k}^2 \quad (13)$$

We turn to the Proca solutions. We must now find  $\mathbf{A}$ ,  $\phi$  is continuous across the plates; that is  $\phi = 0$ ,  $\mathbf{A} = 0$  for  $z < 0$ ,  $z > 2L$  with  $\phi = 0$ ,  $\mathbf{A} = 0$  on the plates themselves.

s-mode:

The mode (9) where  $p$  is given by equations (11, 12) works provided that in place of equation (13) we have

$$\omega_n^2 = p_n^2 + \mathbf{k}^2 + m^2 \quad (14)$$

p-mode:

Now  $\mathbf{A}$  of equation (10) is non-zero on  $z = 0$ . We write

$$\mathbf{A} = \mathbf{A}^T + \mathbf{A}^L \quad (15)$$

with  $\nabla \cdot \mathbf{A}^T = 0$ ,  $\nabla \times \mathbf{A}^L = 0$  and define  $\mathbf{A}^T$  by the right-hand side of equation (10). We then need to look for  $\mathbf{A}^L$ ,  $\phi$ ,  $\nabla \cdot \mathbf{A}^L = i\omega\phi$  such that  $\mathbf{A}$  and  $\phi$  are zero at  $z = 0$  and  $z = 2L$ .

Taking

$$\phi = (i\mathbf{k}^2(\omega^2 - m^2)/\omega^3 p) e^{i\mathbf{k} \cdot \boldsymbol{\rho}} e^{-i\omega t} \sin pz \quad (16)$$

it is not difficult to show that the cosine term in  $\mathbf{A}^L$  cancels with that in  $\mathbf{A}^T$  so that  $\mathbf{A}$  is proportional to  $\sin pz$ . Hence the eigen-

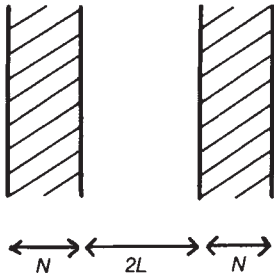


Fig. 1 Reference configuration for the continuum-mode configuration. Shaded area represents conducting plates, each of width  $N$ .

value equation for this mode is also  $\sin(2pL) = 0$  provided that the dispersion relation (14) holds.

Thus the Proca solutions can be constructed out of the corresponding maxwellian solutions. It is easy to show that there are no other solutions with  $\phi, \mathbf{A}$  vanishing within the conducting plates.

### Continuum solutions

We now look for solutions  $\phi, \mathbf{A}^c$  of the Proca equations of frequency  $\omega$  which are non-zero within the conducting plates satisfying

$$i\omega\phi = \nabla \cdot \mathbf{A}^c \quad (17)$$

For convenience we shift the plates to be at  $z = \pm L$  so that we can classify solutions as even and odd. We thus only need to consider the two regions  $0 \leq z \leq L$  and  $z \geq L$  corresponding to the gap and the conductor. Within a perfect conductor  $\mathbf{E} = 0$ . Hence

$$-\nabla\phi + i\omega\mathbf{A}^c = 0 \quad (18)$$

Combining equations (17) and (18) gives

$$\nabla^2\phi + \omega^2\phi = 0 \quad (19a)$$

and hence

$$\nabla^2\mathbf{A}^c + \omega^2\mathbf{A}^c = 0 \quad (19b)$$

In the gap, however, we have from equation (7)

$$\nabla^2\phi + (\omega^2 - m^2)\phi = 0 \quad (20a)$$

$$\nabla^2\mathbf{A}^c - (\omega^2 - m^2)\mathbf{A}^c = 0 \quad (20b)$$

From equations (19) and (20) it appears that although the (group) velocity of the photon in the gap is  $v = (\omega^2 - m^2)^{1/2}/\omega < 1$  (that is,  $v < c$ ),  $v = 1$  (that is,  $v = c$ ) in the conductor. This peculiar result was first noticed by Bass and Schroedinger<sup>23</sup>.

The solutions of equations (19) and (20) are straightforward. First restrict motion to the  $z$ -direction perpendicular to the plates and consider the even solution

$$A_z^c = \cos pz \quad (21a)$$

$$\phi = (ip/\omega) \sin pz \quad (21b)$$

for  $0 < z < L$ ,  $p^2 = \omega^2 - m^2$ , corresponding to a field strength  $E_z = (im^2/\omega) \cos pz$  in the gap. In the conductor  $z > L$  we have

$$A_z^c = A_0 \cos(qz + \delta_e) \quad (22a)$$

$$\phi = iA_0 \sin(qz + \delta_e) \quad (22b)$$

with  $q^2 = \omega^2$  and  $E_z = 0$ . From continuity of  $\mathbf{A}^c$  and  $\phi$  at  $z = L$ , the even phase shift  $\delta_e$  is determined by

$$\tan(qL + \delta_e) = (p/q) \tan pL \quad (23)$$

Similarly the odd solution for frequency  $\omega$  determines the odd phase shift  $\delta_o$  by

$$\tan(qL + \delta_o) = (q/p) \tan pL \quad (24)$$

Clearly, equations (19b) and (20b) for  $A_z^c$  are just the Schroedinger

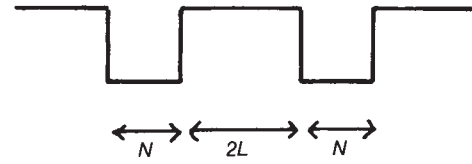


Fig. 2 Equivalent potential well in Schroedinger theory corresponding to the configuration shown in Fig. 1.

ger equation for the scattering of a non-relativistic particle of energy  $E$  and mass  $M$  off a square barrier potential  $V(z) = V_0$ ,  $|z| < L$ ;  $V(z) = 0$ ,  $|z| > L$ . The solutions (21a, b) and (22a, b) are the even and odd solutions to the square well problem with  $p^2 = 2M(E - V_0)$ ,  $q^2 = 2ME$  and the phase shifts  $\delta_e$  and  $\delta_o$  are determined by equations (23) and (24). The boundary conditions of continuity of  $A_z^c$  and  $\phi$  in our case are identical to the Schroedinger conditions of continuity of the wave function and its derivative.

We conclude that an even and an odd continuum-mode solution of the Proca equations exists for any  $p > 0$  with  $\omega^2 = p^2 + m^2$  which has no counterpart in Maxwell theory. It is not difficult to show that the introduction of any momentum  $\mathbf{k}$  parallel to the plates does not change the equations (23) and (24) which determine the phase shifts, except that now

$$\omega_p^2 = \mathbf{k}^2 + p^2 + m^2 = \mathbf{k}^2 + q^2 \quad (25)$$

### Casimir effect

We now consider the quantum-mechanical part of the calculation. To calculate the Casimir force we must obtain the dependence of the total zero point energy of the system on the plate separation  $2L$ . This necessitates a sum over the frequencies of the classical solutions we have found. Again the problem can be separated into the contribution (A) from the maxwellian modes and the contribution (B) from the continuum modes.

The calculation of the contribution from the maxwellian modes is straightforward and has already been performed using dimensional regularization<sup>24,25</sup>. We prefer to use the standard treatment of the effect given in the refs 1, 26. The only change necessary going from  $m = 0$  to  $m \neq 0$  is that the appropriate dispersion relation for the allowed frequencies  $\omega_n$  is given by equation (14) rather than (13). This gives a contribution to the  $L$ -dependent finite part of the zero point energy  $\sum_{n,k} \frac{1}{2} \hbar \omega_n$  of the maxwellian modes of

$$U(L) = -\frac{\pi^2}{3(2L)^3} \int_0^\infty \frac{d\rho(\rho^2 - \mu^2)^{3/2}}{e^{2\pi\rho} - 1} \quad (26)$$

where  $\mu = 2Lm/\pi$ . For  $m = 0$ ,  $\mu = 0$  and the normal expression for the Casimir energy in massless QED is obtained. Approximating equation (26) for small  $\mu$  gives

$$U(L) = \frac{\pi^2}{(2L)^3} \left[ -\frac{1}{720} + \frac{1}{48} \mu^2 - \frac{1}{12} \mu^3 - \frac{1}{16} \mu^4 \ln \mu + O(\mu^4) \right] \quad (27)$$

The first term gives the normal Casimir attractive force. Note that the lowest finite mass correction tends to reduce the effect.

To calculate the contribution from the continuum modes, it is necessary to compute the change in zero point energy for the continuum modes as a result of the gap of length  $2L$  from some reference configuration. It is also necessary to pose the problem in such a way as to ensure that the computation gives a finite answer. All total zero point energies are infinite and, therefore, care is needed. In our problem, the reference configuration for the continuum-mode contribution is best taken to be the situation with the two plates touching (that is,  $L = 0$ ). As the longitudinal modes penetrate the conductors we must also consider the two conducting plates to be of finite width  $N$ . Thus, we consider the modes of the configuration shown in Fig. 1 when compared with the corresponding modes in a solid slab of conductor of total width  $2N$ .



The Schrodinger potential well corresponding to Fig. 1 is the double square well of Fig. 2. As the wells are now attractive there is at least one bound state of the system even for arbitrarily small  $m$ . The solutions for the bound states are obtained in the usual way for  $L=0$  and for  $L \neq 0$ . The even and odd phase shifts are also computed as in Schrodinger theory. The contributions of any bound states and the phase shifts to the zero point energy can now be calculated using methods developed in the plasmon problem<sup>27</sup>. The general expression for the difference between the zero-point energies of the slab system and of the vacuum is

$$V(L, N) = \sum_b \left( -\frac{1}{12\pi} \right) [(m^2 - b^2)^{3/2} - m^3] + \frac{1}{4\pi^2} \int_0^\infty dp p(m^2 + p^2)^{1/2} (\delta_e + \delta_o) \quad (28)$$

where  $\sum_b$  represents a sum over bound modes having  $\omega^2 = k^2 - b^2 + m^2$ , and  $\delta_e, \delta_o$  are the even and odd phase shifts calculated for the double square well (Fig. 2). For the difference  $U = V(L, N) - V(0, N)$  one finds eventually

$$U = \frac{1}{(2L)^3} f(mL, mN) \quad (29)$$

where in the most favourable configuration,  $L \ll N$ , one finds

eventually (for the realistic case  $mL, mN \ll 1$ )

$$f \sim \frac{1}{2\pi^2} (mL)^4 \ln \left[ \frac{N}{4L} \right] \quad (30)$$

This contribution to the Casimir force is, therefore, negligible compared with the leading finite-mass correction to the contribution from the transverse modes.

## Conclusions

The calculations above follow from the uniqueness of the solutions to the Proca equation. The results demonstrate that the Casimir effect is not sensitive to a small photon mass. We have not discussed surface energies here but Aoyama<sup>28</sup> has worked out the surface energy which results for parallel plate geometry in the case of a scalar field of mass  $m$  to be

$$S = \frac{m^3}{18\pi^2} \left( \frac{3\pi}{8} - 1 \right) \quad (31)$$

and the same result is true in our case with this surface energy arising from the continuum modes. There is a corresponding term  $m^3/24\pi$  from the maxwellian modes<sup>20</sup>. So again this effect is smooth as  $m \rightarrow 0$ , as<sup>29</sup> there is no surface energy in the corresponding problem in QED.

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# Anthelic arcs from airborne ice crystals

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*A new set of anthelic arcs resulting from hexagonal, pencil-shaped ice crystals that fall with their axes oriented horizontally is revealed by computer simulation. The arcs result from light rays from the Sun that experience two, and sometimes four, internal reflections in the ice crystal.*

MANY optical effects result from the interaction of sunlight with airborne ice crystals that have the geometrical form of right, hexagonal prisms<sup>1-5</sup>. Most of the well-known halo phenomena occur within  $\sim 50^\circ$  of the Sun but others do occur in different parts of the sky. An area of the sky where several rare effects are found is the region of the anthelic point. (This is the point on the opposite side of the sky from the Sun, at the same elevation above the horizon as the Sun.) Effects that have been reported in the region of the anthelic point include:

(1) Anthelic arcs—oblique arcs crossing the anthelic point. Photographs<sup>1,6,7</sup> and observations of anthelic arcs have been reported<sup>8-14</sup>.

(2) Anhelion—a spot of light at the anthelic point. We know of no photographs that show the anhelion independent of other anthelic phenomena although observations have been reported and mechanisms suggested<sup>1,6,15</sup>.

(3) Anthelic pillar—a column of light extending from slightly above the anthelic point down to the horizon. We know of only one published photograph<sup>1,6</sup> but observations have been reported<sup>11,12</sup>.

(4) Parhelic circle—a circle of light parallel to the horizon passing through the Sun and anthelic point. This effect is understood to result from reflection from the vertical faces of oriented ice crystals<sup>1-4</sup>.

## Computer simulation method

All of the anthelic effects must involve at least one reflection to deflect light to the observer. The parhelic circle involves some rays that are externally reflected from the ice crystals; all other effects appear to involve one or more internal reflections.

R.G.G. *et al.* have developed a simulation method for investigating the origins of ice-crystal optical effects<sup>1,16-18</sup>. For a given

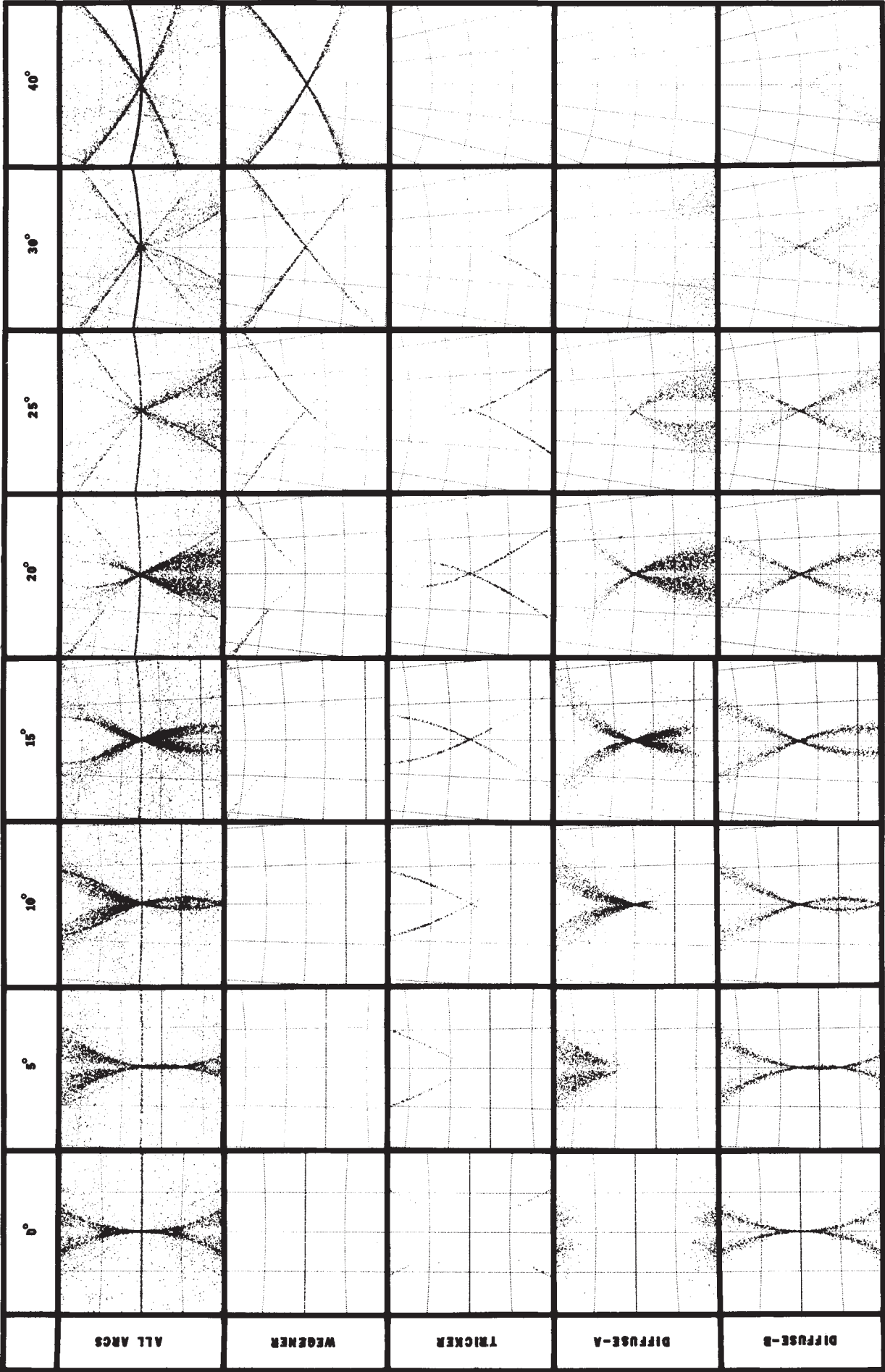
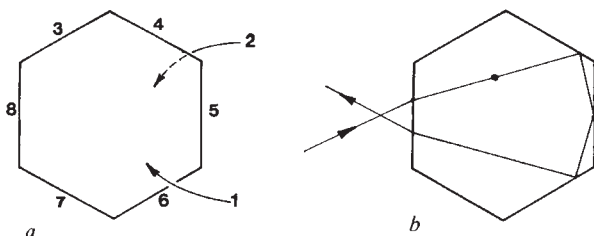


Fig. 1 Intensity patterns that simulate anthelic arcs for various Sun elevations. The grid on each figure consists of lines of constant altitude and of constant elevation, at 10° intervals. The horizon is represented by the darker horizontal line that shows no curvature.





**Fig. 2** *a*, Arbitrary numbering of the faces of a right hexagonal prism, representing an ice crystal. *b*, A ray path through the crystal that can be classified (by reference to *a*) as 814568.

orientation of an ice crystal, they calculate the direction of a light ray from the Sun after it has passed through specified entrance and exit faces of the crystal. This direction is translated into a position in the sky where such a crystal would be located to send the ray to the observer's eye and a spot is plotted to represent that position. For refraction phenomena, which require no internal reflections, their method handles intensity effects fairly well. The loss of intensity due to reflection at each face can be calculated. Another factor accounts for the cross-section of the incident light beam that is transmitted by both the entrance and the exit faces. Analytical expressions for both of these factors can be combined into an overall intensity factor which is used to fix the probability of whether a particular spot will be plotted—a high intensity factor yielding a high probability that the spot will be plotted. The collection of many such spots from a variety of crystal orientations gives a representation of the optical effect predicted from the collection of crystals with the given distribution of orientations and with the specified ray path through the crystal. With this method, however, it is difficult to develop the analytical intensity expression for the restriction in beam cross-section resulting from one or more internal reflections. Mallmann and Greenler used this (Monte Carlo) method to simulate anthelic phenomena<sup>6</sup> ignoring the geometrical intensity factor for the internal reflections.

Pattloch and E.T. have extended the Monte Carlo simulation technique so that these intensity effects can be used for any number of internal reflections<sup>19</sup>. They treat the light as a photon that strikes the oriented crystal at a random location. At each encounter with a crystal face, the photon is either transmitted or reflected (with a probability calculated from the Fresnel reflection coefficients). Its path through the crystal is calculated using geometrical optics and followed until it escapes the crystal and can be represented as a spot on a sky diagram. This Monte Carlo approach is used to obtain the intensities and has significant advantages over the previous method:

- (1) All the reflection and refraction effects that can result from light interacting with a particular crystal shape are obtained in the simulation. Previously, only those preconceived combinations of entrance, exit, and internal reflection faces would have been investigated.
- (2) The relative intensities of all the different arcs and spots are correctly represented for the shape of crystal specified.
- (3) Any number of internal reflections are routinely handled. For the results shown here the calculation includes internal reflections up to 17, any more being negligible.

Additional details of the method are given in ref. 19.

## Results

Figure 1 shows the simulations of anthelic effects for Sun elevations between 0° and 40°. The top row of simulations show everything predicted to occur for columnar (pencil) crystals with their main axes confined to a horizontal plane but with random orientation of the axes in that plane, and random rotational orientation of the crystals about their axes. The ratio of crystal length-to-width is 2.0. The Sun's angular diameter of 0.5° is taken into account by allowing rays to impinge on the crystal from different directions corresponding to different positions on the Sun's disk.



**Fig. 3** Photo of the Wegener anthelic arcs and the parhelic circle for a Sun elevation of 50° taken by A. J. Mallmann in Milwaukee, Wisconsin.

If we look at the result for a Sun elevation of 20° we can see the familiar Wegener<sup>6</sup> arcs, the Tricker arc<sup>6</sup>, and the parhelic circle but there are more structures that are not familiar. To investigate the source of the additional structures, we modified the computer program so that we can identify the path of a ray that is responsible for a given dot. Figure 2*a* shows an arbitrary assignment of numbers to the hexagonal crystal. The side faces are numbered 3 to 8, with the end faces identified as 1 and 2. The path of a ray through the crystal can be classified by a series of numbers beginning with the entrance face. For example, the path of the ray shown in Fig. 2*b* can be classified as 814568.

The intensity plots in the second to fifth row of Fig. 1 show effects from rays taking particular paths through the crystal. These are subsets of the intensity plots of the first row, which includes all of the effects. In each case, we have plotted all of the contributions from rays whose paths are physically equivalent to the one mentioned. For example, the rays producing the Wegener arcs are called the 814 rays but they would include rays such as 315, 416, 725, and all others that enter a side face, reflect off an end face, and exit from another side face located such that there is one face between the entrance and exit faces. For convenience, we refer to such faces as alternate side faces to distinguish them from adjacent side faces or opposite faces.

The second row represents the effect produced by rays taking the path, 814, through the crystal; these are the Wegener arcs.

We have designated as the Tricker arc, the anthelic arc that is produced by rays that enter the end face of a crystal, make two reflections from adjacent or alternate side faces, are reflected back by the opposite end face, and exit from the same end face by which they enter; for example, 18421. See ref. 1 for the argument that for Tricker's mechanism<sup>20</sup> "... any combination of an even number of side-face reflections plus one end-face reflection, in any order, will produce the same set of arcs". We find that rays of the type, 81463, also lie on the same locus and can contribute intensity to the Tricker arc for higher Sun elevations than those paths requiring exit and entrance through the same end face. The third row of Fig. 1 shows the Tricker arc including most of the possible contributions. The shape of the arc is the same as previously predicted<sup>6</sup> but the intensity distribution is altered.

As we identified the types of rays that contributed to intensity in the anthelic region, it was surprising to find that the class of rays that contribute the most intensity for Sun altitudes in the 5°–25° range had not been investigated before. The ray path is shown in Fig. 2*b*. Although it involves four internal reflections, such rays contribute significant intensity in the anthelic region of the plots of Fig. 1. For a Sun elevation of 20°, for example, with a crystal length-to-width ratio of 2.0, the intensity is greater than that from Wegener-arc rays by a factor of 13 and greater

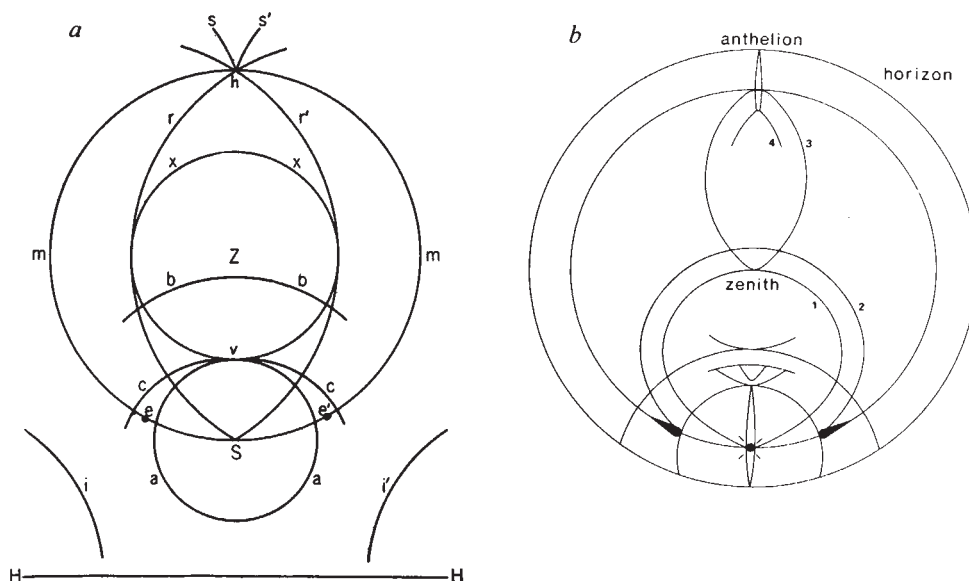


Fig. 4 a, Drawing of a complex display in North Dakota by F. J. Bavendick (ref. 22). b, Drawing of a complex display in Antarctica by J. R. Blake (ref. 11).

than that from the Tricker arc rays by a factor of 7. It is significant, however, that unlike these latter two arcs, which are quite sharp, the pattern produced by the rays of Fig. 2b is much more diffuse. The results are shown in the third row of Fig. 1 labelled diffuse-A.

Another class of rays (8146) also contribute a significant amount of intensity in the anthelic region. The patterns that they produce are shown in the bottom row of Fig. 1, labelled diffuse-B.

These four patterns, together with the parhelic circle, seem to account for all of the intensity patterns that can be seen in the top row. The four sets (and the parhelic circle) account for more than three-quarters of the total number of points for a Sun elevation of 20°. Some ray paths, of course, produce no arcs but add to the general background.

## Discussion

The top row of simulations in Fig. 1 shows our predictions of the anthelic optical effects that result from sunlight interacting with pencil crystals with horizontal axes. It seems reasonable to classify separately only those effects that can be separated visually and identified by observation. The Wegener arcs fulfill this requirement. At Sun elevations above 30° they are the only anthelic arcs present and they can be separated from the general display for lower Sun elevations. Figure 3 shows the Wegener anthelic arcs and the parhelic circle for a Sun altitude of 50°. A few other published photos<sup>1,6,7</sup> confirm their reality.

The Tricker arc is seen most distinctly in our simulations for Sun elevations in the range 20°–30°. The photographic evidence for the Tricker arc is not very convincing. The complex display drawn by Bavendick<sup>21</sup> (Fig. 4a) shows two sets of anthelic arcs that are well represented by the Wegener and Tricker arcs in the simulation for 30°. (Other problems of interpretation are discussed in ref. 1). The simulations seem to show that for some Sun elevations the Tricker arc may be separated visually from the rest of the anthelic potpourri and thus should be separately classified.

For Sun elevations between 0° and 20°, the anthelic display appears to be dominated by the arcs that we have identified as diffuse arcs-A and B. Because these arcs overlap at all Sun elevations, it seems inappropriate to name them separately. Hence, we refer to the combination as the diffuse anthelic arcs.

The intensity comparison of the diffuse arc with the Tricker arc will depend on the aspect ratio of the crystal. Longer pencil crystals should show diffuse arcs that are relatively more intense than the Tricker arcs. Shorter crystals diminish the relative intensities of the diffuse arcs but as the aspect ratio approaches 1, the crystals will no longer orient with horizontal axes.

Mallmann and Greenler's simulation<sup>6</sup> of anthelic effects considered rays (8146) that we show here to produce the diffuse arc-B component. Their results did not show the diffuse arc but showed a concentration at the anthelic point for Sun elevations above 20° and an anthelic pillar for lower Sun elevations. Apparently, the appropriate intensity factors eliminate rays from well-defined areas of the sky and leave the diffuse arcs rather than the general background seen by Mallmann and Greenler.

## Evidence for diffuse arcs

Blake's<sup>11</sup> drawing of the complex sky display he observed in Antarctica in 1958 is reproduced in Fig. 4b. Most features have been reproduced quite satisfactorily by computer simulation for a Sun elevation of 11°. The results are shown in ref. 1. The poorest agreement came in trying to match the Y-shaped structure (labelled 4 in Blake's drawing) with a Tricker arc. The diffuse arc simulation (Fig. 1, Sun elevation of 10°) seems to be a much better match for the structure. (Figure 4b should be inverted for comparison with the simulation of Fig. 1.)

Figure 5 shows a cusp-like arc extending below the anthelic point for a Sun elevation of 21°. The angle between the arms is consistent with that for Tricker's arc. Compared with the simulation for 20° in Fig. 1, the diffuse arcs appear to give a better representation of the width of the arms. Nothing can be seen above the parhelic circle. The simulation does show that the upper part of the diffuse arcs are less intense.



Fig. 5 Photograph of anthelic arcs for a Sun elevation of 21°, taken by R.G.G. at Point Barrow, Alaska.



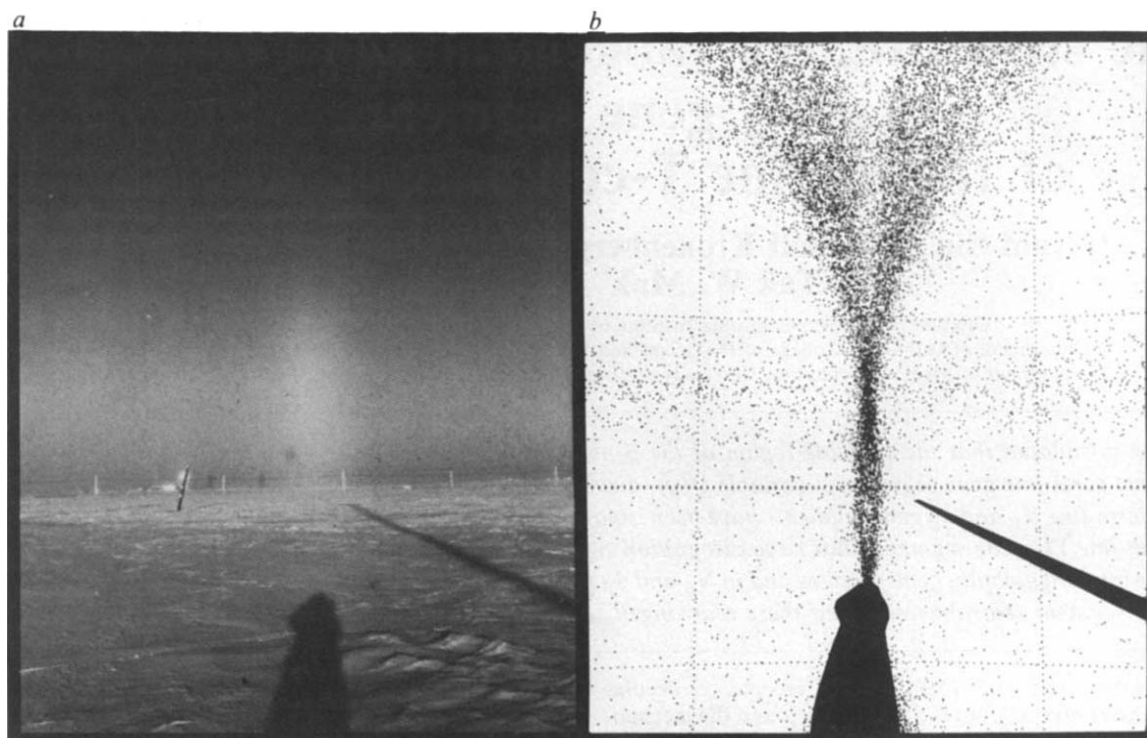


Fig. 6 Photograph (a) of an anhellic pillar with a Sun elevation of  $6^\circ$ , taken by R.G.G. at Point Barrow, Alaska. The simulation (b) has the same scale as the photo.

Pattloch and E.T. have simulated the effects of Hogan's South Pole photograph<sup>19</sup>, in which the structure below the anhellic point is partially obscured by a ghost image of the Sun caused by reflections within the camera lens. The arcs extending above the anhellic point, however, seem to be matched by the combination of diffuse arcs and the Tricker arc.

Figure 6 shows a photo of the anhellic pillar together with an attempt to match it to our simulation for which the pencil crystals have a length-to-width ratio of 1.33, and the crystal axis is permitted to vary by  $2^\circ$  from the horizontal plane. The Sun elevation was  $6^\circ$  during the photo. The most obvious discrepancy between photo and simulation is the absence of the upper portion of the predicted display in the photograph. The photo was taken on 28 March 1978 at Point Barrow, Alaska in the evening. Notes taken during the day indicate that various optical effects appeared and disappeared, but that the lower part of the infralateral arcs was quite persistent. Notes made immediately after the sighting and photographing of the anhellic pillar say: "The pillar appeared to be in the same layer that was giving rise to the ILA [infralateral arcs] all day; at least, the angular height of the effect corresponded to the height of the layer." An earlier note says: "The ILA seems to be formed in a cloud layer about  $5^\circ$  high, where it is brighter than the  $22^\circ$  halo." The

infralateral arcs are produced by the same distribution of pencil-shaped ice crystals that are being considered for these anhellic effects. We would obviously like to have more (and better) photos of these anhellic effects for comparison with our model predictions.

In this simulation, we do not find any specific contribution that produces an anhelion. There are two possibilities. First, the brightness at the anhellic point may just be the superimposed contribution from the various arcs that intersect there. In cases where the arcs are faint, their intersection at the anhellic point may be the only spot bright enough to be seen. Second, the anhelion may exist independently of these other effects but is not caused by the pencil crystals with the orientations we assume here.

## Conclusions

We have used an improved Monte Carlo simulation method to investigate optical effects near the anhellic point that result from sunlight interacting with pencil-shaped ice crystals oriented with their long axes horizontal or nearly horizontal. In addition to the previously described Wegener and Tricker arcs, we find a new set of arcs, the diffuse arcs, that are predicted to dominate the anhellic effects for low Sun elevations.

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# The structure, rearrangement and expression of $D_\beta$ gene segments of the murine T-cell antigen receptor

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*It has been postulated that the variable region of the  $\beta$ -polypeptide of the murine T-cell antigen receptor is encoded by three distinct germ-line gene segments—variable ( $V_\beta$ ), diversity ( $D_\beta$ ) and joining ( $J_\beta$ )—that are rearranged to generate a  $V_\beta$  gene. Germ-line  $V_\beta$  and  $J_\beta$  gene segments have been isolated previously. Here we report the isolation and characterization of two germ-line  $D_\beta$  gene segments that have recognition signals for DNA rearrangement strikingly similar to those found in the three immunoglobulin gene families and in  $V_\beta$  and  $J_\beta$  gene segments. The  $D_\beta$  and  $J_\beta$  segments can join in the absence of  $V_\beta$  gene segment rearrangement and these rearranged sequences are transcribed in some T cells.*

IMMUNOGLOBULINS and T-cell antigen receptor molecules exhibit extensive diversity but the genetic basis and the mechanisms responsible for generating this diversity are well understood only for the immunoglobulin genes<sup>1–3</sup>. Immunoglobulins are heterodimers made up of disulphide-bridged light and heavy chains each of which is divided into variable regions that recognize antigen, and constant regions that control effector functions. Genes that encode the variable region of light chains are created by a DNA rearrangement that joins two distinct gene segments—variable ( $V_L$ ) and joining ( $J_L$ )<sup>4–6</sup>. The variable region genes of heavy chains are created by the joining of three distinct gene segments—variable ( $V_H$ ), diversity ( $D_H$ ), and joining ( $J_H$ )<sup>7,8</sup>. One mechanism for the generation of immunoglobulin diversity is joining of different  $V_H$ ,  $D_H$ , and  $J_H$ , or  $V_L$  and  $J_L$  gene segments<sup>9,10</sup>. Also gene segments may be joined together at different sites, leading to a junctional variability<sup>10–12</sup> and in the heavy chain gene family, extra nucleotides, not found in  $V_H$ ,  $D_H$ , or  $J_H$  gene segments, may be added between these gene segments as a result of the joining process (N-region diversity)<sup>11,13</sup>. The 3' side of the  $V$  gene segments, the 5' side of the  $J$  gene segments, and both sides of the  $D$  gene segments are flanked by recognition signals for DNA rearrangements. These signals comprise three components—a highly conserved palindromic heptamer, a nonconserved spacer sequence of 12 or 23 nucleotides, and a relatively conserved A/T-rich nonamer<sup>6–8,14</sup>. It is believed that these recognition sequences play a critical role in DNA rearrangements because they are present in all three of the immunoglobulin gene families and, therefore, have been conserved throughout vertebrate evolution.

The T-cell antigen receptor is a heterodimer composed of two disulphide-bonded polypeptides,  $\alpha$  and  $\beta$ , that have a similar molecular weight<sup>15–17</sup>. The genes that encode the  $\beta$ -chain of the T-cell antigen receptor have recently been identified and they share many features with those encoding immunoglobulins. The nucleotide sequence of cDNA clones encoding the  $\beta$ -chain of the T-cell receptor from both mouse and man contains regions similar to  $V$  and  $J$  gene segments and  $C$  genes<sup>18–20</sup>. Distinct germ-line  $V_\beta$  and  $J_\beta$  gene segments and  $C_\beta$  genes have been isolated from genomic libraries<sup>21–23</sup>. Two highly similar and closely-linked  $C_\beta$  genes denoted  $C_{\beta 1}$  and  $C_{\beta 2}$  have been isolated on a single cosmid clone of germ-line DNA and each  $C_\beta$  gene has a cluster of  $J_\beta$  gene segments associated with it<sup>23</sup>. The  $V_\beta$  and  $J_\beta$  gene segments have recognition signals for DNA rearrangements that are very similar to those of their immunoglobulin counterparts<sup>21–23</sup>.

Both murine and human  $\beta$  cDNA clones encoding a complete variable region contain eight or more nucleotides than their

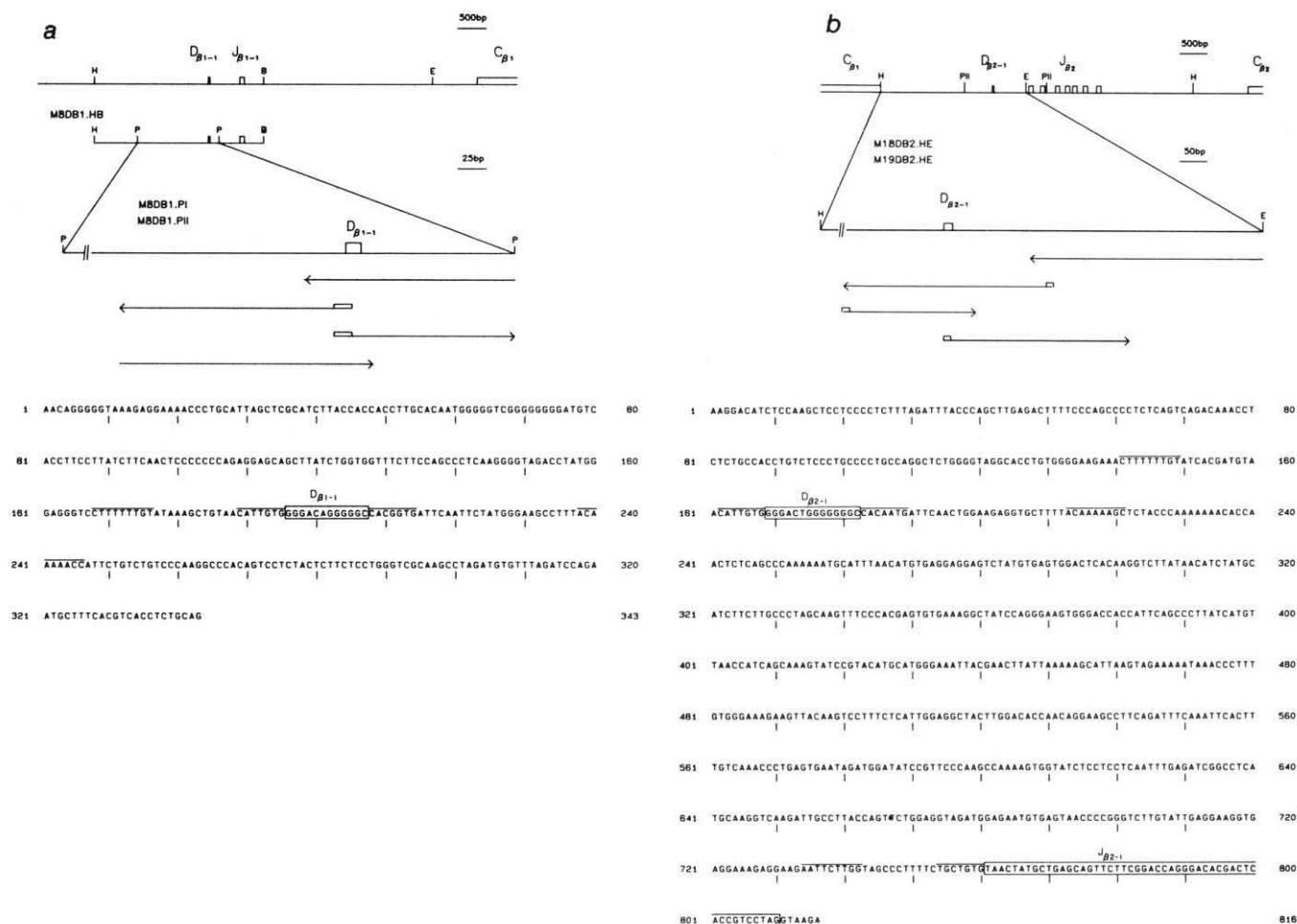
corresponding germ-line  $V_\beta$  and  $J_\beta$  gene segments suggesting that a portion of the  $\beta$  variable region is encoded by a third gene segment analogous to the  $D_H$  gene segment of immunoglobulins<sup>21,22</sup>. Indeed, in this paper we present the structure of two germ-line  $D_\beta$  gene segments. One of these  $D_\beta$  gene segments lies to the 5' side of the  $J_{\beta 1}$  gene cluster and the second lies to the 5' side of the  $J_{\beta 2}$  gene cluster, between  $C_{\beta 1}$  and  $J_{\beta 2}$ . Recognition signals for DNA rearrangements on either side of these  $D$  gene segments are described, as are rearrangements and transcripts involving the  $D_\beta$  gene segments.

## $D_\beta$ gene segment of each $J_\beta$ gene cluster

The partial nucleotide sequences of four murine  $\beta$ -chain cDNA clones have been published<sup>19</sup>. Two of them appeared to lack a  $V_\beta$  gene segment but contain joined  $D_\beta$ – $J_\beta$  sequences. This conclusion was based on several features. First, the published sequences 5' to the  $J_\beta$  gene segments resemble neither  $V_\beta$  gene segments nor the sequences found 5' to the germ-line  $J_\beta$  gene segments. Second, in both of these clones the sequences 5' to the joined  $J_\beta$  gene segment have a recognition signal for DNA joining that includes the heptamer, a 12-nucleotide spacer sequence, and the A/T-rich nonamer sequence, as well as a short sequence that could be a  $D_\beta$  coding segment located between the joining signal and the  $J_\beta$  gene segment. Two 40-base oligonucleotides complementary to a portion of these putative  $D_\beta$  gene segments and their 5' flanking sequence were synthesized using the technique of Horvath *et al.*<sup>24</sup>. The oligonucleotide T5 is complementary to a region starting at nucleotide position 56 and ending at position 95 of the published sequence of the cDNA clone 86T5 (ref. 19). T6 is complementary to the cDNA clone TM86 at positions 15–54 (ref. 19). We have hybridized these oligonucleotides to Southern blots of a genomic cosmid clone known to contain the  $J_{\beta 1}$  and  $J_{\beta 2}$  gene clusters. T6 hybridizes most strongly to a restriction fragment located to the 5' side of the  $J_{\beta 2}$  gene cluster, while T5 hybridizes more strongly to the region 5' to the  $J_{\beta 1}$  gene cluster (G.S., unpublished observations). We have determined the nucleotide sequence in the vicinity of the hybridizing DNA fragments and have linked them by restriction mapping and DNA sequencing to their respective  $J_\beta$  gene clusters (Fig. 1 and unpublished results).

Each of these hybridizing regions appears to contain a  $D_\beta$  gene segment by the following criteria (Fig. 1). (1) There is a 5' recognition sequence for DNA joining with a 12-base pair spacer sequence. (2) There is a 3' recognition sequence for DNA joining with a 23-base pair spacer. Both of these sequences are similar to their immunoglobulin counterparts (Fig. 2). (3) One of these sequences, or a highly similar  $D_\beta$  gene segment, appears





**Fig. 1** Nucleotide sequences of germ-line  $D_{\beta 1-1}$  and  $D_{\beta 2-1}$  gene segments. **a**, The  $D_{\beta 1-1}$  subclones sequencing strategy and sequence. **b**, The  $D_{\beta 2-1}$  subclones sequencing strategy and sequence. The sequence from position 410 to position 844 had been previously determined on both strands<sup>23</sup>. The coding regions of the  $D_{\beta}$  and  $J_{\beta}$  joining segments are boxed, and the 5' and 3' heptamer and nonamer recognition signals are overlined. Sequences were obtained by subcloning restriction fragments into the vectors M13mp8, M13mp18 and M13mp19 (ref. 47) and sequenced using the dideoxynucleotide method<sup>48</sup>, as modified by E. Strauss (unpublished). Synthetic oligonucleotides complementary to mouse genomic sequences were utilized as sequencing primers in some cases. The positions of these primers are indicated by the open box at the ends of the arrows. Restriction enzyme sites are abbreviated as follows: B, *Bam*HI; E, *Eco*RI; H, *Hind*III; P, *Pst*I; PII, *Pvu*II.

to form part of the variable region of the 86T1 $\beta$  cDNA clone<sup>19</sup>. In addition, the coding sequences for these  $D_{\beta}$  gene segments are similar to some immunoglobulin  $D_H$  gene segments (Fig. 2a). We will denote the  $D$  gene segment to the 5' side of the  $J_{\beta 1}$  gene cluster as  $D_{\beta 1-1}$  and the  $D$  gene segment to the 5' side of the  $J_{\beta 2}$  gene cluster as  $D_{\beta 2-1}$ . The  $D_{\beta 1-1}$  gene segment is located 647 base pairs (bp) upstream from the 5'-most germ-line  $J_{\beta 1}$  gene segment (Fig. 1a; M.K., R.H., E.S., unpublished results), while the  $D_{\beta 2-1}$  gene segment is located 578 bp upstream from the 5'-most germ-line  $J_{\beta 2}$  gene segment (Fig. 1b). Because the murine immunoglobulin heavy chain locus also has a  $D_H$  gene segment,  $D_{Q52}$ , found 696 bp 5' to its  $J$  gene cluster<sup>11</sup>, there may be some functional significance of a single  $D$  gene segment closely linked to the  $J$  gene cluster in the germline.

There probably are additional  $D_{\beta}$  gene segments that have not yet been located in the germ-line DNA. With the exception of three nucleotides missing from the 3' end, the coding and 5' flanking sequences of the  $D_{\beta 2-1}$  gene segment are identical to the  $D_{\beta}$  gene segment expressed in the TM86 cDNA clone. We believe, therefore, that this cDNA clone includes the  $D_{\beta 2-1}$  gene segment. The 86T5 cDNA  $D_{\beta}$  gene segment is also shorter than the  $D_{\beta 1-1}$  gene segment by three base pairs, and in addition it has one difference at its 3' end and one in the 5' flanking region out of the 90 base pairs compared. It is therefore possible that the 86T5  $D_{\beta}$  gene segment is derived from a different germ-line gene; comparable similarity between members of a family is

seen in several immunoglobulin  $D_H$  gene segment families<sup>25</sup>. Alternatively, somatic mutation or polymorphism between inbred mouse strains could account for these differences. In addition, the  $D_{\beta}$  gene segment in 2B4.71, a cDNA clone from a helper T-cell hybridoma<sup>21</sup> specific for cytochrome c, is different from the three other  $D_{\beta}$  gene segments. Thus the two  $D_{\beta}$  gene segments identified each lie to the 5' side of their corresponding  $J_{\beta}$  gene segment clusters and it appears likely that additional  $D_{\beta}$  gene segments will lie to the 5' side of the  $J_{\beta 1}$  gene cluster.

### Structure of germ-line $D_{\beta}$

Figure 2 shows the germ-line  $D_{\beta 1-1}$  and  $D_{\beta 2-1}$  coding and flanking sequences aligned with each other, with the  $D_{\beta}$  sequences from several  $\beta$ -chain cDNA clones, and with some representative sequences from the immunoglobulin heavy chain gene family. The  $D_{\beta 1-1}$  gene segment has 12 nucleotides while the  $D_{\beta 2-1}$  gene segment has 14 nucleotides (Fig. 2a) and they therefore resemble murine  $D_H$  segments, which range in size from 10–23 nucleotides<sup>25</sup>. The sequences of both the  $D_{\beta 1-1}$  and  $D_{\beta 2-1}$  gene segments are G-rich and they are similar at 10 out of 12 nucleotides compared; they are also similar to the presumed  $D_H$  gene segment found in the heavy chain of some oxazolone-binding immunoglobulins<sup>26</sup>, and to the  $D_H$  region found in the heavy chain of the 18-81A-2 Abelson murine leukaemia virus (A-MuLV)-transformed cell line<sup>27</sup> (Fig. 2a). It has been

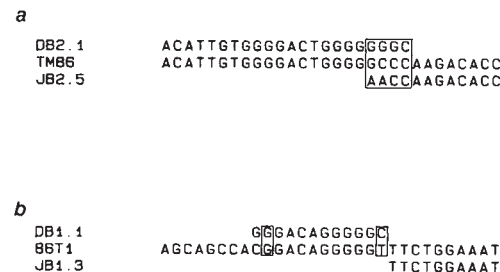


**Fig. 2** A comparison of the  $D_\beta$  coding and flanking sequences with those found in immunoglobulin gene segments. **a**, Comparison of  $D_\beta$  and  $D_H$  gene segment coding regions. 86T5 and 86T1 are from  $\beta$  cDNA clones derived from thymus RNA<sup>19</sup>; TM86 is a cDNA clone from the 3.3T hybridoma<sup>19</sup>; D2B4 is derived from a cDNA clone from a cytochrome c-specific T-helper hybridoma<sup>21</sup>; oxazalone and D18-81A-2 are  $D_H$  coding sequences deduced from the sequence of immunoglobulin heavy chain mRNAs<sup>26,40</sup>; Q52, SP2.3, FL16.1 are germline  $D_H$  segments<sup>11,12,25</sup>. **b**, Comparison of  $D_\beta$  and  $D_H$  gene segment 5' flanking regions. **c**, Comparison of  $D_\beta$  and  $V_H$  gene segment 3' flanking regions. The flanking sequence of four unrelated  $V_H$  gene segments that were aligned to each other are shown. The sequences are from the indicated references: V11 (ref. 49), V441 (ref. 50), V108A (ref. 51) and V23 (ref. 52). V2B4 is the  $V_\beta$  region gene from a  $\beta$ -chain cDNA clone from a cytochrome c-specific helper-T hybridoma<sup>21</sup>. Boxed sequences represent the nonamer and heptamer recognition signals for DNA rearrangement. Gaps were inserted to maximize homology.

previously observed that the  $D_\beta$  gene segment in the 2B4.71 cDNA clone is similar to the immunoglobulin  $D_H$  gene segment  $D_{Q52}$  (ref. 21). It has also been observed that the coding and flanking regions of the mouse and human  $D_{\beta 1-1}$  gene segments are highly conserved<sup>28</sup>. The significance of the similarity observed between the mouse  $D_\beta$  and  $D_H$  gene segments is unclear. Figure 2b shows the 5' flanking regions of the  $D_{\beta 1-1}$  and  $D_{\beta 2-1}$  gene segments aligned with each other, with the 86T5 and TM86  $\beta$  cDNA clones and with representative members of four murine  $D_H$  gene segment families<sup>25</sup>. Figure 2c shows the aligned 3' flanking sequences of the  $D_{\beta 1-1}$  and  $D_{\beta 2-1}$  gene segments. These 3' flanking sequences are also aligned with immunoglobulin  $V_H$  sequences as opposed to  $D_H$  gene segments, since the  $V_H$  gene segments also have 23-base pair spacers in their 3' joining signals. Gaps were inserted to maximize the similarity between the sequences. The 5' flanking sequences of the  $D_{\beta 1-1}$  and  $D_{\beta 2-1}$  gene segments are similar at 35 out of 47 positions compared. The  $D_{\beta 1-1}$  and  $D_{\beta 2-1}$  3' flanking sequences share 31 out of 60 nucleotides compared. Comparison of the far-flanking regions of the  $D_{\beta 1-1}$  and  $D_{\beta 2-1}$  gene segments indicates that they share the highest similarity at the recognition signals. Aside from these signals, these flanking regions have little sequence similarity with either the 5' flanking region of  $D_H$  gene segments or the 3' flanking region of  $V_H$  gene segments.

### $D_\beta$ recognition signals

As was noted by Early *et al.*<sup>7</sup>, the 12 and 23 nucleotide spacer sequences of immunoglobulin joining recognition sequences correspond to approximately one and two turns of the DNA helix, respectively. An interesting feature of the recognition sequences for immunoglobulin  $V_H$  and  $V_L$  gene rearrangements is that the one-turn recognition sequence is always joined to a



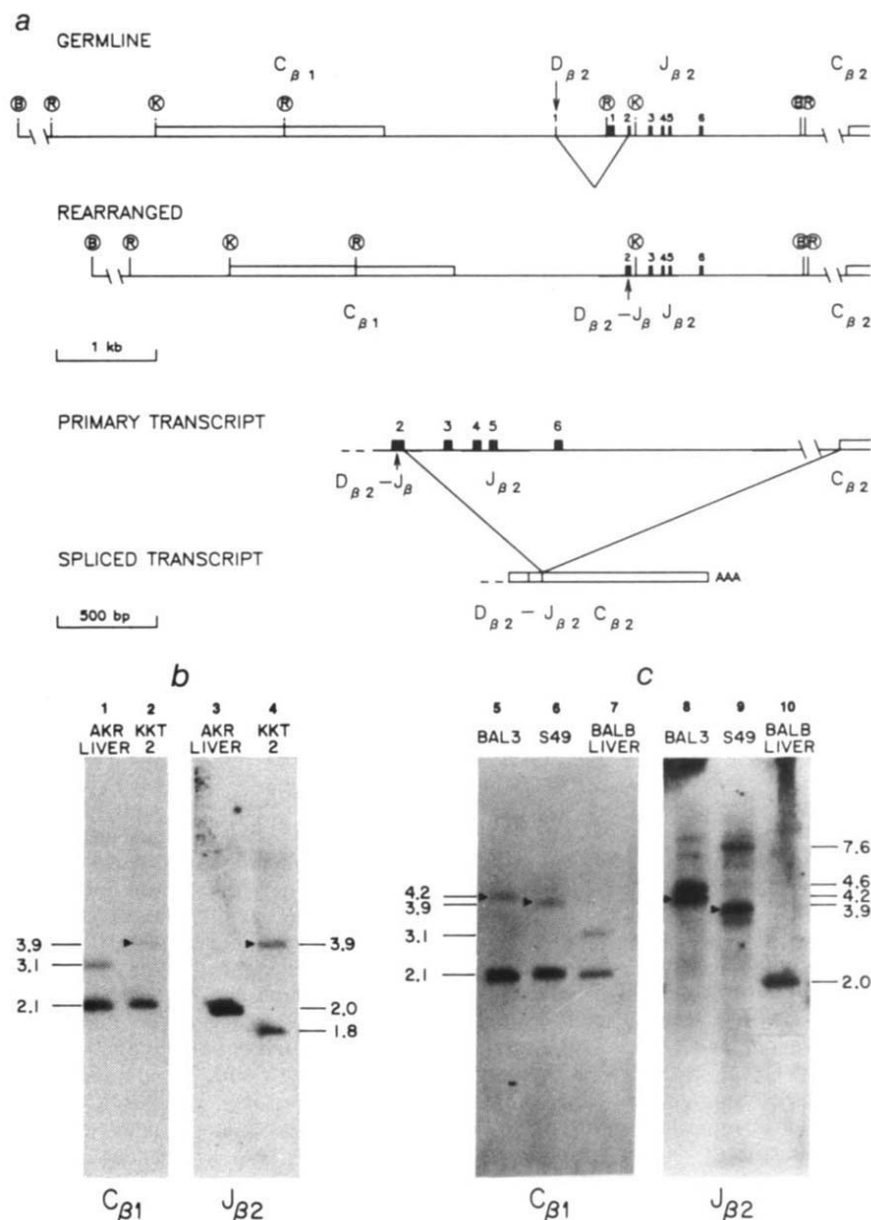
**Fig. 3** Junctional and N-region diversity in  $\beta$ -chain rearrangement. Boxed regions indicate gene segment junctions. **a**, The cDNA clone TM86, compared with germline gene segments  $D_{\beta 2-1}$  and  $J_{\beta 2-5}$ . The last three nucleotides of  $D_{\beta 2-1}$  and the first two nucleotides of  $J_{\beta 2-5}$  have been deleted in the joining event, and one nucleotide of TM86 (a C at position 20) is not encoded by either germline gene segment. **b**, The cDNA clone 86T1, compared with germline gene segments  $D_{\beta 1-1}$  and  $J_{\beta 1-3}$ . One nucleotide at the 5' end of the  $D_{\beta 1-1}$  gene segment has been deleted or substituted in the  $D_\beta$ - $J_\beta$  joining. One nucleotide of 86T1 (a T at position 20) is not encoded by either the  $D_{\beta 1-1}$  or  $J_{\beta 1-3}$  gene segments. No nucleotides were deleted from the  $J_{\beta 1-3}$  gene segment during the  $D_\beta$ - $J_\beta$  joining event; the nucleotide 5' of the first T is a G present in the joining sequence.

two-turn recognition sequence. This rule of one- and two-turn joining is followed in the  $\beta$ -gene family of T-cell receptors as well (Fig. 2b, c)<sup>21-23</sup>. The  $V_\beta$  and  $J_\beta$  gene segments have two-turn and one-turn recognition signals, respectively, and the  $D_\beta$  gene segment has a one-turn recognition signal to its 5' side and a two-turn recognition signal to its 3' side. This organization of recognition sequences permits several types of  $V_\beta$  gene formation consistent with the one- to two-turn joining rule:  $V_\beta$  gene segments may join directly to  $J_\beta$  gene segments,  $V_\beta$ - $D_\beta$ - $J_\beta$  joining may occur involving a single  $D$  gene segment, or two or more  $D_\beta$  gene segments could join between the  $V_\beta$  and  $J_\beta$  gene segments. Thus, the organization of the recognition sequences for the  $\beta$ -gene family should permit additional combinatorial joining possibilities to be employed in the generation of  $V_\beta$  diversity.

### Junctional and N-region diversity

Junctional diversity in immunoglobulin variable regions is caused both by the imprecise joining of  $V_L$  and  $J_L$  or  $V_H$ ,  $D_H$ , and  $J_H$  gene segments that can lead to the deletion of nucleotides from the ends of these gene segments<sup>10-12</sup> and by the addition of nucleotides at the junction between  $V_H$  and  $D_H$  or  $D_H$  and  $J_H$  gene segments that are not found in either of the germ-line counterparts (N-region diversity)<sup>11,13</sup>. Analysis of the coding regions of germ-line  $D_\beta$  and  $J_\beta$  gene segments indicates that both mechanisms for generating junctional diversity occur in the  $\beta$ -chain of the T-cell receptor. Figure 3a compares the published sequence of the TM86 cDNA clone<sup>19</sup>, which contains the  $D_{\beta 2-1}$  sequence joined to the  $J_{\beta 2-5}$  gene segment, to the germ-line sequences of  $D_{\beta 2-1}$  and  $J_{\beta 2-5}$ . In the TM86 cDNA three bases have been deleted from the 3' end of the  $D_{\beta 2-1}$  gene segment, and two bases have been deleted from the 5' end of the  $J_{\beta 2-5}$  gene segment. In addition, N-region diversity is indicated by the presence of a cytidine nucleotide not found at the end of either germ-line gene segment that is present at the  $D$ - $J$  boundary. A second possible example of both types of diversity can be found by comparing the published sequence of the 86T1 cDNA clone<sup>19</sup> that contains a complete variable region gene segment joined to the  $D_{\beta 1-1}$  and  $J_{\beta 1-3}$  (M.K. and R.H., unpublished) gene segments (Fig. 3b). Although the central ten base pairs of the 86T1  $D_\beta$  gene segment are identical to the germ-line  $D_{\beta 1-1}$  sequence, the end nucleotides have been altered. The first nucleotide of the  $D_{\beta 1-1}$  gene segment at the  $V_\beta$ - $D_\beta$  boundary has been deleted or substituted, while the last nucleotide at the  $D_\beta$ - $J_\beta$  boundary has been replaced with a different base. In this latter example, it is also possible that no deletions or additions occur, but instead a  $D_\beta$  gene segment that is slightly different





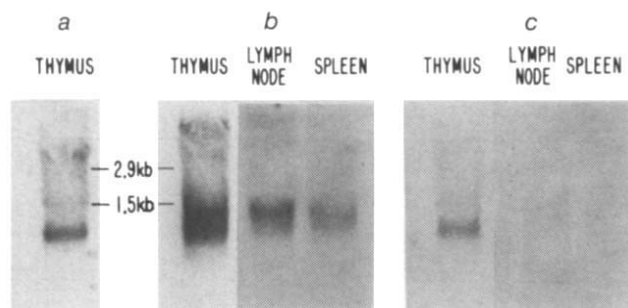
**Fig. 4**  $D_{\beta}$ - $J_{\beta}$  joining in T-cell tumour DNA. Southern blots<sup>43</sup> of *Eco*RI digested DNA were hybridized with a  $C_{\beta 1}$  cDNA clone<sup>54</sup> and a fragment from a germ-line cosmid clone that contains  $J_{\beta 2}$  sequences<sup>23</sup>. Conditions used for the Southern blots have been described previously<sup>55</sup>. **a**, A model for the rearrangement and expression of the partially joined ( $D_{\beta}$ - $J_{\beta}$ ) genes. The  $C_{\beta}$  genes are depicted as open boxes; the separate exons of these genes are not shown. The  $D_{\beta 2}$  and  $J_{\beta 2}$  gene segments are numbered and are shown as vertical lines or filled boxes. Some relevant restriction sites for the Southern blot analysis are indicated. B, *Bam*HI; K, *Kpn*I; E, *Eco*RI. The rearrangement presented in the figure joins the  $D_{\beta 2-1}$  to the  $J_{\beta 2-2}$  gene segment eliminating the third *Eco*RI site as well as  $J_{\beta 2-1}$  and leading to the rearranged fragments described in the text. Rearrangement to other  $J_{\beta 2}$  gene segments is also possible. The scale used to depict the transcripts is expanded twofold. The dotted lines at the 5' end reflect uncertainty about the structure of this part of the transcript. **b**, Hybridization of the  $C_{\beta 1}$  cDNA (lanes 1, 2) and  $J_{\beta 2}$  fragment (lanes 3, 4) to AKR strain tumour KKT-2 and to AKR liver DNA. **c**, Hybridization of the  $C_{\beta 1}$  (lanes 5-7) and  $J_{\beta 2}$  (lanes 8-10) probes with DNA from the BALB/c tumours BAL3 and S49 and with BALB/c liver DNA. In both **b** and **c** the filter was first hybridized with  $C_{\beta 1}$  the probe was removed, and the same filter was then hybridized with the  $J_{\beta 2}$  probe. The arrows denote the identically-sized rearranged fragment that could be detected by both probes. The estimated sizes in kilobases of the hybridizing fragments are indicated.

from the  $D_{\beta 1-1}$  gene segment may be present in 86T1. It is also possible that inter-strain sequence polymorphisms can account for these differences, since the germ-line sequences are from B10 mice and in this case the cDNA sequence is from BALB/c mice. Because the only differences are at the boundaries between the joined gene segments, we consider these latter possibilities to be less likely.

### $D_{\beta}$ - $J_{\beta}$ joining without $V$ - $D$ - $J$ joining

A productive DNA rearrangement is defined as one in which the  $V$ ,  $D$  and  $J$  (or  $V$ - $J$ ) joining process occurs in such a way that a functional polypeptide chain can be expressed. In immunoglobulin genes a variety of nonproductive rearrangements may occur, including incomplete rearrangements of  $D_H$  and  $J_H$  gene segments joined together without a  $V_H$  gene segment. Such incomplete heavy chain rearrangements occur frequently in immature B cells<sup>29,30</sup> but are also found in some mature B cells<sup>11,31</sup> and T cells as well<sup>12</sup>, and are thought to be the first step in the assembly of heavy-chain variable region genes. The  $\beta$ -gene segments may undergo  $D$ - $J$  rearrangements in the absence of  $V_{\beta}$  joining in a manner similar to their immunoglobulin counterparts. We have characterized the  $\beta$ -gene rearrangements in a large number of T-cell lines, hybridomas, and tumours (M.K., J. Goverman, M. Malissen,

R.H., manuscript in preparation) and have observed several cases in which the DNA from T-cell tumours have undergone an apparent  $D_{\beta}$ - $J_{\beta}$  joining. For example, when germ-line DNA is digested with the restriction enzyme *Eco*RI and hybridized with a  $C_{\beta 1}$  cDNA probe, there are two hybridizing fragments of 2.1 and 3.1 kilobases (kb). The 3.1 kb fragment is at the 3' end of the  $C_{\beta}$  gene beginning close to the translational stop codon and ending just 5' to the first  $J_{\beta 2}$  gene segment<sup>23</sup> (Fig. 4a). In the T-cell tumours S49, BAL3, and KKT-2, the 3.1 kb fragment at the 3' end of the  $C_{\beta 1}$  gene is replaced by a larger fragment that is either 3.9 kb (S49 and KKT-2) or 4.2 kb (BAL3) (Fig. 4b, c). Hybridization of the same filters with the  $J_{\beta 2}$  probe reveals two rearranged fragments in each case, including one fragment identical in size to the one detected with the  $C_{\beta 1}$  probe. Southern blots of DNA from these tumours were generated from DNA digested with *Bam*HI and hybridized with the  $C_{\beta 1}$  and  $J_{\beta 2}$  probes. Digestion of germ-line DNA with this enzyme will generate a single fragment that should hybridize with both probes (Fig. 4a). In the DNA from each of the tumours one of the fragments hybridizing with the  $J_{\beta 2}$  probe and the only fragment hybridizing with the  $C_{\beta 1}$  cDNA are identically sized and approximately 1,000 bp smaller than the germ-line fragment (M.K., R.H., unpublished observations). We therefore conclude that the rearrangements 3' to the  $C_{\beta 1}$  gene in these tumours are small deletions. Since this deletion spans the *Eco*RI site just to



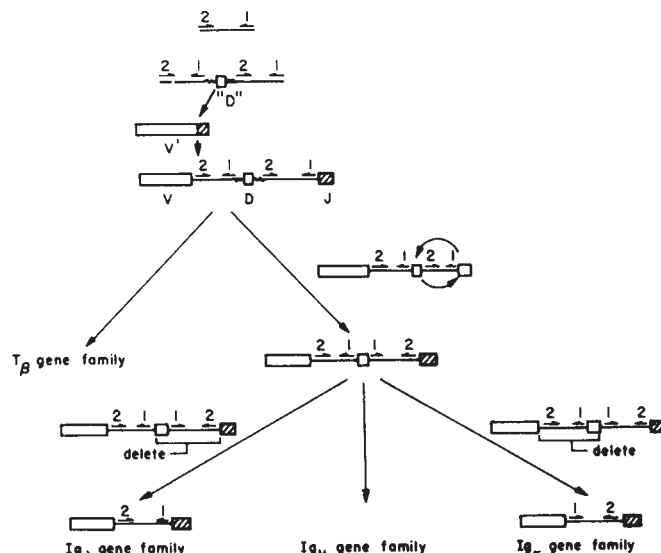
**Fig. 5** Hybridization of  $D_\beta$  and  $C_\beta$  probes to RNA from lymphoid tissues. The probes are as follows: *a*, T6 synthetic oligonucleotide; *b*,  $C_\beta$  cDNA clone; *c*, T5 synthetic oligonucleotide. RNA was prepared from thymus, spleen, and lymph node of 4-week-old DBA 1/J female mice by homogenization of the tissues in guanidinium thiocyanate followed by centrifugation through a cushion of  $\text{CsCl}^{56}$ . Northern blots of total cell RNA and hybridization with a  $C_\beta$  probe were performed as described previously<sup>57</sup>. Hybridization with oligonucleotides was done at room temperature with end-labelled synthetic probe at a concentration of  $0.5 \text{ pmol ml}^{-1}$ . The blots were washed in  $2 \times \text{SSC}/0.1\% \text{ SDS}$  at room temperature followed by washing in the same solution at  $37^\circ\text{C}$ . One filter was used in *a*, *b* and *c*; following hybridization and exposure to film, the probe was removed by stringent washing before retesting with a second probe. The migration distance of *Escherichia coli* ribosomal RNA molecular weight markers is indicated. Other markers included mouse 28 + 18S ribosomal RNA (4.8 and 1.9 kb) and MS2 phage RNA (3.6 kb).

the 5' side of the  $J_{\beta 2}$  gene cluster, the most likely explanation for these events is that the  $D_{\beta 2-1}$  gene segment has joined to a  $J_{\beta 2}$  gene segment (Fig. 4a). The slight difference in the size of the rearranged fragment in different tumours could reflect joining to different  $J_{\beta 2}$  gene segments. We have similar evidence that  $D_{\beta 1-1}$  to  $J_{\beta 1}$  joining events can occur as well (unpublished data). Thus it appears likely that the  $D_{\beta 1-1}$  and  $D_{\beta 2-1}$  gene segments do participate in both complete ( $V_\beta$ - $D_\beta$ - $J_\beta$ ) and partial ( $D_\beta$ - $J_\beta$ ) DNA rearrangements. By analogy with immunoglobulin heavy-chain genes, these partial rearrangements may represent the first step in  $\beta$ -chain variable region gene assembly.

### $D_\beta$ - $J_\beta$ transcription not using $V_\beta$ promoters

Northern blot analyses have been carried out on RNA from thymus, lymph node, and spleen using both a  $C_\beta$  probe (Fig. 5b) and the oligonucleotides T5 and T6 (Fig. 5a, c). The  $C_\beta$  probe detects two species, approximately 1.3 and 1.0 kb in size, when hybridized to total RNA from the thymus. Similar results have been obtained with poly(A)<sup>+</sup> RNA (M.K., G.S., unpublished observations). Spleen and lymph node RNA have a single  $C_\beta$  hybridizing transcript of 1.3 kb. When RNA from these three lymphoid tissues is hybridized with either the T5 or T6 oligonucleotide, only the 1.0 kb thymus RNA is detected. It is important to note that both T5 and T6 are complementary to 6 bases of  $D_\beta$  coding sequences and 34 bases of 5' flanking sequence. These two probes therefore do not hybridize with fully rearranged  $V_\beta$ - $D_\beta$ - $J_\beta$  genes since these rearrangements eliminate the 5'  $D_\beta$  flanking sequence. Because the probes also do not cross-hybridize with each other, we conclude that at least two  $D_\beta$  gene segments contain 5' promoters that permit expression in thymus: one is the  $D_{\beta 2-1}$  gene segment and the second is the  $D_{\beta 1-1}$  gene segment or a highly similar  $D$  gene segment such as the one found in the 86T5 cDNA clone. It has recently been demonstrated that each immunoglobulin  $D_H$  gene segment also has a promoter located in the 5' flanking DNA<sup>32</sup>.

We have hybridized both the  $C_\beta$  probe and the  $D_\beta$  oligonucleotide probes to RNA from the S49 and KKT-2 tumours that are believed to have joined  $D_{\beta 2}$ - $J_{\beta 2}$  gene segments. The KKT-2 tumour has only a 1.0 kb transcript that hybridizes with both



**Fig. 6** A model for the evolution of the immunoglobulin and  $\beta$  T-cell receptor gene families. The number 1 represents the one-turn recognition signal; the number 2 represents the two-turn recognition signal.

the T6  $D_\beta$  oligonucleotide and a probe specific for the 3' end of  $C_{\beta 2}$ . Several other T-cell tumours and T-cell hybridomas contain 1.0 kb transcripts that hybridize with both  $C_\beta$  probes and the T6 oligonucleotide. However, RNA from S49 does not hybridize with either oligonucleotide, and it contains a 1.3-kb but not a 1.0-kb transcript when hybridized with the  $C_\beta$  cDNA probe (M.K., unpublished observations).

Based on the blot-hybridization and nucleotide sequence data presented here and the partial nucleotide sequences of the TM86 and 86T5 cDNA clones, we have presented a model for the rearrangement and expression of the  $D_\beta$  gene segments and their 5' flanking sequences (Fig. 5a).  $D_\beta$ - $J_\beta$  rearrangement may stimulate transcription from a promoter located 5' to several of the  $D_\beta$  gene segments. This transcript is 1.0 kb long, polyadenylated, and contains a joined  $D_\beta$ - $J_\beta$  gene segment spliced to the exons normally used in the  $C_\beta$  gene. Although we cannot formally rule out transcription from this promoter in the absence of gene rearrangement, it should be noted that the KKT-2 tumour has no germ-line  $J_{\beta 2}$  clusters (Fig. 4c) and that a transcript derived from a promoter 5' to the  $D_{\beta 2-1}$  gene segment that includes the intron between  $D_{\beta 2-1}$  and  $J_{\beta 2-1}$  as well as the exons of  $C_{\beta 2}$  would be at least 1.6 kb. No transcript of this length has been detected with any of the probes employed. The prevalence of the 1.0 kb transcript in thymus, as opposed to lymph node and spleen, would suggest that many of the immature cells in this tissue have undergone a partial ( $D_\beta$ - $J_\beta$ ) rearrangement. However, as some functional mature T lymphocytes have a 1.0-kb transcript that hybridizes with the T6 oligonucleotide (M.K., unpublished), there is probably a low level of 1.0-kb transcript in lymph node and spleen that could not be detected on our blots.

Immunoglobulin gene expression in B lymphocytes is thought to result from gene rearrangements that bring into proximity two widely separated elements: a promoter sequence located 5' to the  $V$  gene segments<sup>33,34</sup> and an enhancer sequence located in the intron between the  $J$  gene segments and the  $C$  genes<sup>35-39</sup>. In the case of the  $D_\beta$ - $J_\beta$  transcript, however, the location of the promoter relative to any postulated enhancer would not be greatly affected by the relatively small deletions involved in the  $D_\beta$ - $J_\beta$  joining we have described. Therefore the means by which  $D_\beta$ - $J_\beta$  joining might stimulate transcription are unknown. Furthermore, Northern blots of RNA from the S49 tumour indicate that rearrangement is necessary but may not be sufficient for transcription. Currently, we are further characterizing the  $D_\beta$ - $J_\beta$



DNA rearrangements and  $D_\beta$  transcripts in a number of T-cell tumours in order to understand better the relationship between the rearrangements and the 1.0 kb  $C_\beta$  transcripts that we have described.

### Are $D_\beta$ - $J_\beta$ transcripts translated?

In the immunoglobulin gene families, transcripts have been described that originate from unrearranged  $\kappa^{40}$  and heavy-chain genes<sup>41</sup> or from incompletely rearranged ( $D_H$ - $J_H$ ) heavy-chain gene segments<sup>27,41</sup>. Some of the RNA molecules do not give rise to detectable polypeptides and are referred to as sterile transcripts<sup>27,42</sup>. These sterile transcripts might not be physiologically significant, or else the RNA molecule itself might have some regulatory function. Similar speculations apply to the 1.0-kb transcripts containing  $D_\beta$  gene segments. In addition, it is also possible that these  $D_\beta$ - $J_\beta$  transcripts encode functional polypeptides. If the 1.0-kb transcripts use the entire  $C_\beta$  gene and the same 3' end as is found in the 1.3-kb  $C_\beta$  RNA, then about 100–200 nucleotides remain for the  $D_\beta$  coding region and its 5' flanking sequence. The nucleotide sequences flanking the  $D_{\beta 1-1}$  and  $D_{\beta 2-1}$  gene segments have open reading frames for more than 50 codons. A methionine codon that is in frame with the germ-line  $D_\beta$  gene segments is located 5' to both  $D_{\beta 1-1}$  and  $D_{\beta 2-1}$  (data not drawn for  $D_{\beta 2-1}$ ). Moreover, the  $D_{\beta 1-1}$  flanking sequence has a signal sequence for membrane insertion of a nascent polypeptide<sup>43</sup> 136 nucleotides 5' to the  $D_\beta$  gene segment (Fig. 1b). A possible signal sequence is also located following the methionine codon 5' of  $D_{\beta 2-1}$  (data not shown). Alt and co-workers have demonstrated that most immunoglobulin  $D_H$  gene segments have open reading frames in their 5' flanking sequence that begin with a methionine codon followed by a hydrophobic leader sequence. They have also demonstrated that transcripts derived from partial  $D_H$ - $J_H$  rearrangements give rise to truncated  $C_\mu$ -containing polypeptides<sup>32</sup>. We therefore believe that the  $D_\beta$ - $J_\beta$  transcripts will in at least some cases give rise to truncated  $\beta$  polypeptides. The function, if any, of these truncated polypeptides is unknown. Because it has been proposed that the synthesis of complete heavy chain and light chain polypeptides derived from productively rearranged genes prevents further V gene rearrangement<sup>40,44</sup>, we might speculate that the truncated  $C_\mu$  and  $C_\beta$  polypeptides will in a similar fashion somehow stimulate or facilitate the V gene rearrangement process. It is also possible that these truncated proteins provide a stimulatory signal for cell growth similar to that provided by antigen bound to the intact receptor. Alternatively they might function at the cell surface with regard to the development of the repertoire of receptors via network interactions.

### Evolution of gene rearrangements

It has been proposed that the ability of the antibody and T-cell receptor gene families to undergo DNA rearrangements evolved from the insertion of a transposable element into a primordial uninterrupted V gene exon<sup>6,45</sup> (Fig. 6). This theory proposed the existence of a transposon with asymmetric inverted repeats similar to those found in the joining sequences of the contemporary antigen-receptor gene families. If inserted in the genome, duplication of this transposon along with a short stretch of flanking sequence would lead to a more complex transposon containing a proto- $D$  gene segment (Fig. 6). Subsequent insertion of this transposon into a primordial V gene would generate a gene segment organization, at least with respect to joining sequences, that is similar to that of the  $\beta$  genes. Duplication of this locus would lead to two separate gene families: the  $\beta$ -chain gene family and the immunoglobulin gene family. As shown in Fig. 6, an inversion bounded by the two right-hand inverted repeats could generate the precursor of the heavy chain gene family. As outlined previously<sup>45</sup>, further duplications of this heavy chain gene precursor could have occurred to create precursors of all three immunoglobulin gene families. A deletion on the right hand or 3' end of the transposon as indicated in Fig. 6 generates the proto- $\lambda$  gene family with its organization

of joining signals. This may have occurred via homologous recombination between these signals. A similar deletion at the 5' end could give rise to the  $\kappa$  gene family.

This model can account for some of the unique features of variable region genes. The known genes encoding antigen receptors on B and T lymphocytes, cell-surface molecules of the major histocompatibility complex (MHC),  $\beta_2$ -microglobulin and some other cell-surface proteins associated with the immune system, are believed to have evolved in part from a primordial exon that encoded an immunoglobulin-like domain<sup>46</sup>. With the exception of the variable region genes, each domain in these proteins having an immunoglobulin structure is encoded by a single exon. The insertion of a transposon into a primordial variable region gene can account for the fact that all the V gene families are split into several exons at a similar location. The theory accounts for the presence of a  $D$  gene segment in only two of the four antigen-receptor gene families and also explains the different configurations of DNA rearrangement signals in these families. While the evolutionary history outlined above implies that the  $\beta$ -chain gene family arose first, several other pathways beginning with insertion of a slightly different transposon are also possible<sup>45</sup>. Finally, this theory proposes a relatively simple explanation for the evolution of the DNA rearrangement signals in that it proposed these signals evolved from the inverted terminal repeats of a mobile DNA sequence but it remains unclear how these signals and the proteins that recognize them became adapted for the highly regulated rearrangements of the immune system.

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**Note added in proof:** While this paper was in the press, the sequence of the  $D_{\beta 1-1}$  gene segment and the presence of this gene segment in the 86T1 and 86T5 cDNA clones was reported by Kavalier *et al.*<sup>58</sup>.

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# Apparent discontinuous transcription of *Trypanosoma brucei* variant surface antigen genes

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*The repeated mini-exon sequence that encodes the first 35 base pairs of all variant surface antigen mRNAs of Trypanosoma brucei directs the synthesis of a discrete 137-nucleotide transcript. It thus seems that variant surface antigen mRNAs are transcribed discontinuously, and we present two alternative models for how this might occur.*

THE ability of African trypanosomes to establish chronic infections in their mammalian hosts depends on a highly developed system of antigenic variation, whereby individual members of the parasite population change the composition of their surface coat<sup>1–3</sup>. This antigenic variation is controlled at the level of gene expression<sup>4,5</sup>, each trypanosome possessing a large repertoire of genes (estimated at over 100; refs 6, 7) coding for the antigenically distinct variant surface glycoproteins (VSGs) which comprise the coat. At any one time and on any one trypanosome, only one species of VSG can be detected<sup>8</sup>, suggesting that mutual exclusion operates between the VSG genes. Activation of VSG genes occurs by a two-step process requiring first the duplication and transposition of a silent basic copy (BC) of the gene into one of a few telomeric expression sites by a process equivalent to gene conversion, then selection of the expression site over others for transcription<sup>9–15</sup>. The extra copy of the gene thus produced is called the expression-linked copy (ELC). The so-called 'non-duplication-activated' VSG genes described by others probably represent genes which had already undergone the first step in activation (that is, gene conversion into an expression site) before the variants were isolated for study. Although the structure of expression sites and the sequences involved in the gene conversion event have been identified for some variants<sup>16–19</sup>, little is known about the second step, particularly how activation occurs and how the mutual exclusion operates between the different expression sites.

Recently, a major clue to this problem has come from the finding that the 5' 35 nucleotides of VSG mRNAs are not contiguously encoded with the protein-coding portion of the gene<sup>3</sup>, and that this spliced leader segment is identical for different VSG mRNAs regardless of which telomeric expression site they occupy<sup>14</sup>. The genomic location of the 35-base pair (bp) mini-exon coding for the spliced leader has recently been reported to be a 1.35-kilobase pair (kb) segment tandemly repeated 100–200 times (as one or more clusters) at an unidentified locus in the genome but at least 30 kb upstream of the active VSG gene<sup>20,21</sup>. Two other important observations concerning the spliced leader are that it is detected on many RNA molecules of varying size in blot analyses using RNA from

different life stage forms of *Trypanosoma brucei* (ref. 21 and M. Parsons *et al.*, personal communication) and that homologous sequences have been detected in the genomes of related species and genera<sup>22</sup>.

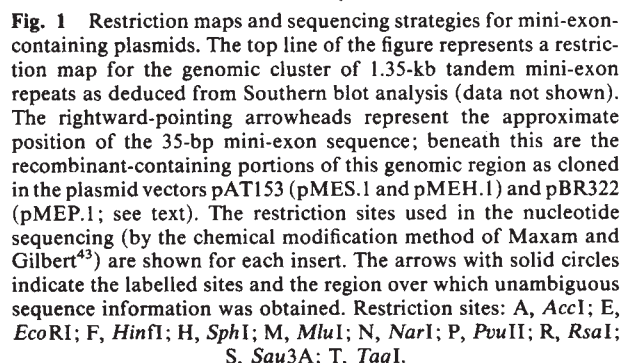
Several models have been proposed to explain how a contiguous mRNA is produced from such an unusual arrangement of exons<sup>20–23</sup> and how the activity of different expression sites is regulated. These have included chromosome end exchange with splicing of very long transcripts, and discontinuous transcription whereby the mini-exon and ELC are flanked by their own initiation and termination sites. To determine how VSG mRNAs are produced and processed, we have studied the 1.35-kb repeats and identified their transcriptional products.

## Molecular cloning of a mini-exon repeat

To enable nucleotide sequence analysis of the mini-exon repeat, recombinant plasmids containing individual repeats were generated. This was initially done using a *PvuII* digest of the genomic DNA of *T. brucei* as this enzyme has been reported to release the mini-exon-containing portion of the repeat as a discrete band at ~720 bp<sup>21</sup>. Such a band was excised from an agarose gel, the DNA purified by electroelution<sup>24</sup> and ligated into the *PvuII* site of the plasmid vector pBR322 (ref. 25). A sample of this same excised material was radiolabelled by nick-translation<sup>26</sup> and used as a probe in colony hybridization<sup>27</sup>, thereby identifying recombinants containing repetitive DNA. Four plasmids thus identified were further characterized and three were found to contain indistinguishable inserts with at least one *RsaI* site. As the mini-exon should include an *RsaI* site, nucleotide sequence analysis in the vicinity of this site was performed on one of the plasmids (pMEP.1). The strategy and sequence obtained are shown in Figs 1 and 2, respectively. This demonstrated unambiguously that pMEP.1 contains a portion of the mini-exon repeat.

To obtain a recombinant plasmid containing an insert representative of the complete 1.35-kb repeat, a ligation was performed using the 1.35-kb fraction of trypanosome genomic DNA digested with *Sau3A* and the plasmid vector pAT153 (ref. 28) digested with *BamHI*. Recombinants were screened with the





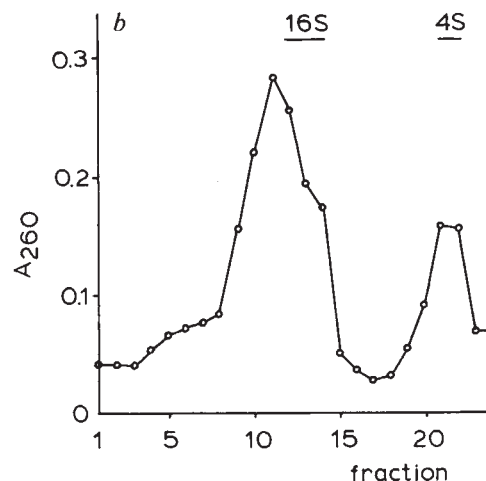
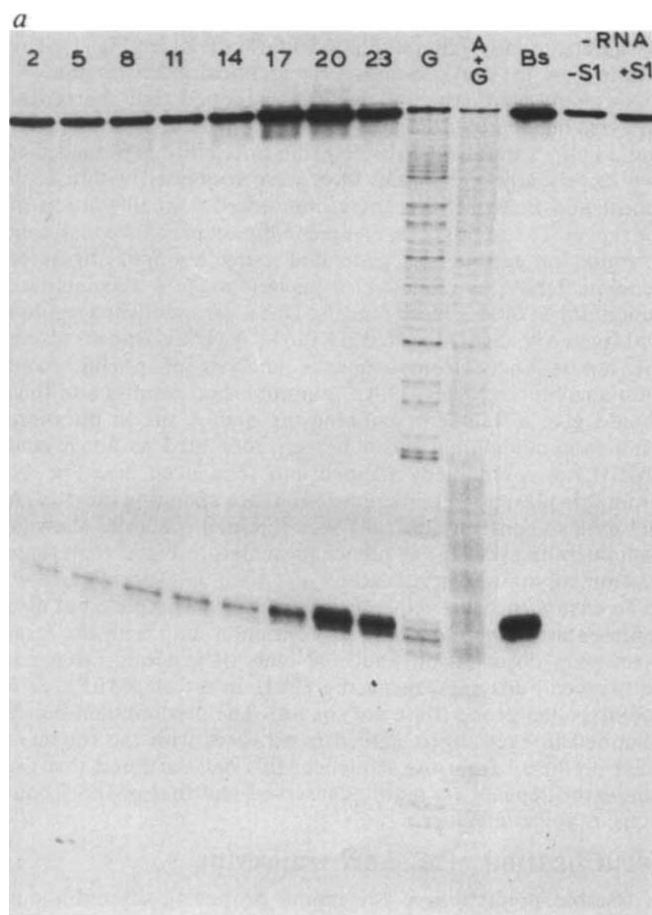
**Fig. 1** Restriction maps and sequencing strategies for mini-exon-containing plasmids. The top line of the figure represents a restriction map for the genomic cluster of 1.35-kb tandem mini-exon repeats as deduced from Southern blot analysis (data not shown). The rightward-pointing arrowheads represent the approximate position of the 35-bp mini-exon sequence; beneath this are the recombinant-containing portions of this genomic region as cloned in the plasmid vectors pATI53 (pMES.1 and pMEH.1) and pBR322 (pMEP.1; see text). The restriction sites used in the nucleotide sequencing (by the chemical modification method of Maxam and Gilbert<sup>43</sup>) are shown for each insert. The arrows with solid circles indicate the labelled sites and the region over which unambiguous sequence information was obtained. Restriction sites: A, *AccI*; E, *EcoRI*; F, *HinfI*; H, *SphI*; M, *MluI*; N, *NarI*; P, *PvuII*; R, *RsaI*; S, *Sau3A*; T, *TaqI*.

**Fig. 2** Nucleotide sequence of a complete 1.35-kb mini-exon repeat. The nucleotide sequence of the sense strand of pMES.1 is presented with numbering from the 5'-end of the 35-bp mini-exon, which is boxed. The restriction sites used in the cloning (see Fig. 1) are underlined and annotated. The *Sau*3A site (GATC) used in cloning the insert is duplicated at the beginning and end of the sequence. The thickly underlined segment represents the 17-nucleotide sequence for which a complementary oligonucleotide was synthesized and used in subsequent experiments (see Figs 3 and 5). The arrow between positions 137 and 138 indicates the 3'-terminus of mini-exon-derived RNA as described in the text.

Using the recombinant plasmid pMES.1 and the strategy shown in Fig. 1, the complete nucleotide sequence of one repeat unit containing the mini-exon was determined. This is presented in Fig. 2 with position +1 being the first nucleotide of the mini-

To ensure that this sequence was not only complete but also representative, we compared its restriction map with the fragment sizes observed on Southern blots of genomic DNA cut with several enzymes, using the *Pvu*II insert of pMEP.1 as a radiolabelled probe (data not shown). The predominant bands obtained in each digest agreed in all cases with the fragment sizes predicted from the sequence; this demonstrated that the mini-exon repeats are highly conserved and that pMES.1 contains a typical member.

This result could be interpreted as protection by a short RNA of 137 nucleotides derived from the mini-exon repeats and/or by a longer transcript with a discontinuity at this position in the RNA/DNA duplex. To discriminate between these two alternatives, two procedures were used. The first examined the  $S_1$ -protection by different size fractions of RNA produced by sucrose-gradient centrifugation (Fig. 3*b*). Maximal protection was found in fractions 19–21, which possessed RNA with a peak size slightly larger than 4S RNA (that is, ~100–200 nucleotides), with diminishing but detectable protection in the remaining fractions (Fig. 3*a*). This strongly suggested that the protection observed with total RNA was due, at least in part, to the presence of a small RNA. The protection observed with other fractions, particularly those containing larger RNA, may be due



**Fig. 3** *a*,  $S_1$  nuclease mapping of the 3'-terminus of medRNA. Lanes 2–23 are reactions performed with RNA from pooled sucrose gradient fractions, as described in *b* below. Next to these are two marker sequencing reactions<sup>43</sup> on the labelled probe. The lane labelled Bs contains the result of a protection experiment with 50  $\mu$ g of total RNA. The two controls, which contain no added RNA, are with (+S1) or without (–S1)  $S_1$ -nuclease, respectively. *b*,  $A_{260}$  profile of total trypanosome RNA fractionated by sucrose gradient centrifugation. The size markers are based on samples from each fraction analysed by electrophoresis and ethidium bromide staining (not shown).

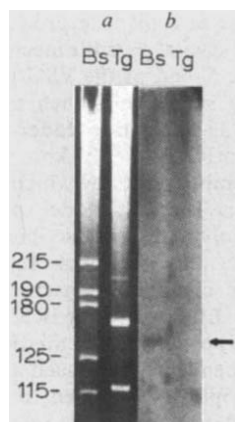
**Methods:** *a*, A [ $3'$ - $^{32}$ P]-end-labelled *Pvu*II digest of pMES.1 was re-cut with *Eco*RI to generate a uniquely end-labelled fragment corresponding to positions +59 to +183 plus 375 bp of the vector (see Fig. 1).  $S_1$  protection was done essentially as described elsewhere<sup>30</sup>. Briefly, the probe was mixed with different fractions of bloodstream-form RNA, precipitated, redissolved and denatured in 80% formamide at 80 °C for 10 min. Renaturation of RNA/DNA hybrids was promoted by incubating at 45 °C for 3 h in the same buffer, before diluting with 10 vol of aqueous buffer, cooling to 30 °C and adding 35 units of  $S_1$  nuclease. Digestion was for 30 min, after which the reactions were phenol-extracted, isopropanol-precipitated, resuspended in formamide loading buffer<sup>43</sup> and resolved by PAGE in a 6% polyacrylamide gel with 7 M urea<sup>44</sup>. *b*, A 15–30% linear sucrose gradient was prepared in 0.1 M NaCl, 1 mM EDTA, 20 mM Tris-HCl (pH 7.5), 0.1% SDS. About 1 mg of total trypanosome RNA was overlaid with the same buffer and the samples spun in an SW-41 rotor (Beckman) at 40,000 r.p.m. at 20 °C for 9.5 h. Fractions were collected from the bottom of the tube and the  $A_{260}$  measured. For the  $S_1$ -protection experiments, 5% of each sample was removed and pooled in groups of three (numbered by the middle fraction).

to contamination with the small RNA. Alternatively, they may be a result of protection by hybrid transcripts comprising the small RNA at their 5' ends linked to RNA from, for example, the ELC region, by the mechanisms discussed below.

The second method to detect and size transcripts from the mini-exon repeat used Northern blot analysis<sup>31</sup>. For this procedure, total trypanosome RNA was resolved by polyacrylamide gel electrophoresis (PAGE) in denaturing conditions, transferred to GeneScreen membrane<sup>32</sup> and hybridized with a [ $5'$ - $^{32}$ P]-labelled, synthetic oligonucleotide complementary to positions +117 to +133. This 17-mer was chosen because it excluded the mini-exon itself, which was known to give intense hybridization throughout RNA blots when used as a probe<sup>21</sup> and because the  $S_1$ -protection experiments indicated that it should hybridize to mini-exon-derived transcripts. Figure 4a shows the ethidium bromide stain of a polyacrylamide/7 M urea gel in which the major small RNA species is readily apparent. Figure 4b presents the autoradiogram produced after hybridizing a GeneScreen transfer of a duplicate half of this gel with the 17-mer; only one band is detected in the track containing trypanosome RNA and no signal is observed in the track containing RNA from the unrelated parasitic protozoan, *Toxoplasma gondii*. The size of the band is ~140 nucleotides based on published values for the small ribosomal RNAs of trypanosomes<sup>33</sup>, thus confirming that the mini-exon repeats are transcribed to yield a short, discrete RNA which we have termed mini-exon-derived RNA (medRNA). With regard to its precise size, this result is, within experimental error, consistent with medRNA being 137 nucleotides long. We cannot, however, exclude the possibility that medRNA is as many as 4–5 nucleotides longer and that  $S_1$  nuclease nonspecifically digested the 3' end of the RNA/DNA hybrid. Note, however, that the nucleotides at positions 138 and 140 are cytosines which, as CG pairs, would be less susceptible to such action. A definite answer awaits 3'-sequence analysis of medRNA.

These results also suggest that the 5'-terminus of medRNA is at about position +1 in the mini-exon repeat. To confirm this, we used the synthetic oligonucleotide from the RNA blot analyses as a primer for reverse transcriptase extensions of medRNA. The result (Fig. 5) shows a major band co-migrating with a heterologous DNA marker of 135 nucleotides. Using a different heterologous marker (a *Hind*III digest of bacteriophage  $\lambda$  DNA), the size of this band was estimated as 133 nucleotides. This slight discrepancy is probably due to differences in base composition. Allowing for this variation, the extension has an approximate length of  $134 \pm 1$  nucleotides. Given that the 5'-end of the primer represents position +133 of the mini-exon repeat, this result confirms that the 5'-end of medRNA corresponds to position +1, the same as that found for the spliced leader of mature VSG mRNAs<sup>13,14</sup>. The minor band in Fig. 5 at  $138 \pm 1$  nucleotides may be an artefact of reverse transcriptase (for example, loop-back synthesis) or it may be indicative of a minor RNA 4 nucleotides longer than the major species. Such minor heterogeneity might be due to variation in the mini-exon repeat sequences and/or in the site of transcriptional initiation. No other major bands were observed in other loadings of this sample run for longer or shorter periods (data not shown).





**Fig. 4** Detection of the mini-exon-derived RNA. *a*, Ethidium bromide stain of electrophoretically separated trypanosome RNA. Lane Bs contains 10  $\mu$ g of total RNA from bloodstream-forms of *T. brucei* (strain MITat 1.4). Lane Tg contains 10  $\mu$ g of RNA from *T. gondii* as a negative control. The major bands in lane Bs are polysome-derived and their sizes in nucleotides are taken from ref. 33. The two major bands in the *T. gondii* RNA are presumably 5S and 5.8S. *b*, Autoradiograph of an RNA blot of the other half of the gel shown in *a*, containing identical samples, and having been probed with [ $5'$ - $^{32}$ P]-labelled 17-mer complementary to positions +117 to +133 of Fig. 2.

**Methods:** *a*, Total RNA was separated on an 8% polyacrylamide gel (1.5 mm thick) containing 7 M urea<sup>44</sup>. *b*, The electrophoresed RNA was electroblotted to GeneScreen membrane<sup>32</sup> at 4 V for 18 h at 4 °C in 25 mM Na phosphate (pH 6.5). The filter was prehybridized in 50% formamide, 5 $\times$ SSC (1 $\times$ SSC = 0.15 M NaCl, 0.015 M Na citrate), 1 $\times$ Denhardt's, 25 mM Na phosphate (pH 6.5), 0.1% SDS, 0.1% Na pyrophosphate, 100  $\mu$ g ml<sup>-1</sup> denatured calf thymus DNA at 30 °C for 2 h. A synthetic oligonucleotide complementary to positions +117 to +133 (see Fig. 2) was synthesized by standard means, [ $5'$ - $^{32}$ P]-labelled by polynucleotide kinase, added to the prehybridization solution and the incubation continued with gentle shaking for 15 h at 30 °C, followed by 5 h at 22 °C. The filter was washed in four changes of 2 $\times$ SSC, 0.5% SDS for 15 min each at 22 °C. Autoradiography was at -70 °C with an intensifying screen for 6 days.

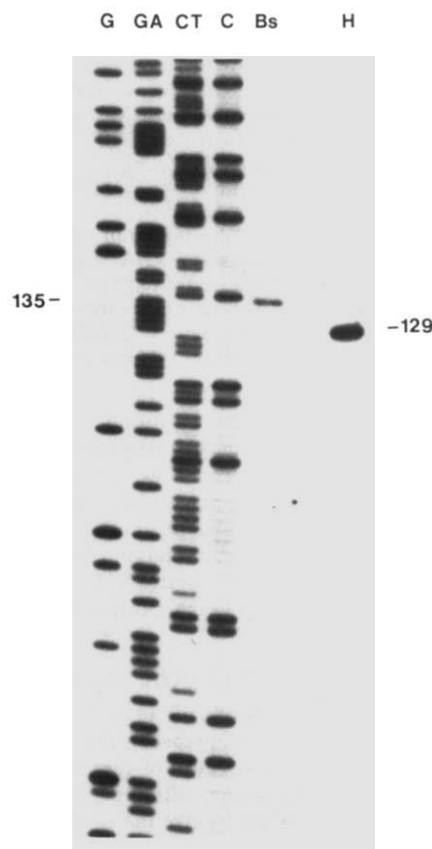
## Conclusions

We have cloned and sequenced a complete copy of the 1.35-kb mini-exon repeat of *T. brucei* and identified a short RNA of 137 nucleotides as its transcriptional product. The data indicate that this repeat is a typical member of a highly conserved family—this is demonstrated further by the virtually complete agreement of the sequence presented in Fig. 2 with the partial sequence for the region from -130 to +61 recently published elsewhere<sup>21</sup>; the four differences (three single base changes, one insertion) are all localized to between -115 and -125. As the sequences in both cases were determined from two plasmids, the differences may be indicative of minor, but real, variation in the repeats.

In addition to the region coding for medRNA, each 1.35-kb mini-exon repeat contains about 1,200 nucleotides whose function is not clear; part of this region is presumably involved in the regulation of medRNA expression, but the remainder could be involved in some other aspect of trypanosome gene expression. Of particular note is the repetitive nature of the sequence around the *Sau*3A site, consisting of 1-, 2-, 4- and 6-nucleotide repeats (T, AT/AC, ATTT/GTTT and AACTC, respectively). It will be interesting to determine whether these regions of the repeat are as exactly conserved as that coding for the medRNA.

The predicted sequence of medRNA can be drawn with several alternative regions of intramolecular base-pairing (data not shown). This suggests that medRNA might adopt a stable conformation with substantial secondary structure, but, as we have no data on RNase sensitivity, we cannot present a reliable structure.

The detection of medRNA raises questions about the signals directing its transcription. As noted by De Lange *et al.*<sup>21</sup>, the

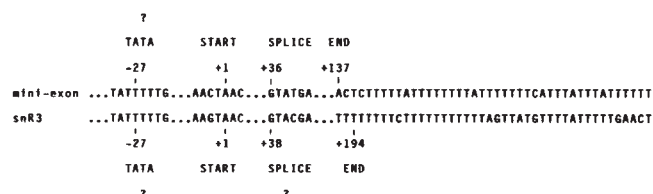


**Fig. 5** Identification of the 5'-terminus of the medRNA. Polyacrylamide gel electrophoresis of the reverse transcriptase product primed off medRNA (lane Bs). Marker tracks contained standard sequencing reactions<sup>43</sup> of a mouse cDNA clone (provided by J. Kavaler; lanes G, A + G, C + T and T) or a [ $3'$ - $^{32}$ P]-labelled *Hind*III digest of  $\lambda$  DNA (lane H).

**Methods:** The [ $5'$ - $^{32}$ P]-labelled synthetic oligonucleotide described in Fig. 4 legend used as a primer in extension reactions using AMV reverse transcriptase. About 4  $\mu$ g of primer were hybridized to 20  $\mu$ g of total bloodstream-form RNA in 1.2 M Na phosphate buffer (pH 6.8), 0.5% SDS, 12.5 mM EDTA at 21 °C for 4 h. After ethanol precipitation, the reverse transcriptase reaction was done in 50 mM Tris-HCl (pH 8.3), 6 mM MgCl<sub>2</sub>, 1 mM dithiothreitol, 60 mM KCl, 0.1 mM dATP, dCTP, dTTP and dGTP, and AMV reverse transcriptase (50 units) at 37 °C for 1 h. The sample was ethanol-precipitated, resuspended in formamide loading dye and electrophoresed on a 7 M urea, 8% polyacrylamide gel. The dried gel was autoradiographed at -70 °C for 72 h.

octanucleotide centred at position -27 upstream of the putative initiation site for transcription consists of TATTTTGTG, similar to the consensus sequence generally found at this position in eukaryotic RNA polymerase II promoters (the so-called TATA box; see ref. 34). Assuming trypanosomes are usual in their types and functions of RNA polymerase and given that the medRNA ultimately forms the 5'-end of the VSG mRNA, the finding of a putative TATA box at this position is expected. Further studies on relative drug sensitivities and 5'-cap structures are needed to confirm medRNA as an RNA polymerase II transcript. The termination site cannot be compared with the general case as too few sequences of eukaryotic transcriptional terminators have been reported to enable consensus sequences to be made.

Recently, the complete sequence of a small nuclear RNA (snR3) and its gene have been reported in yeast<sup>32,35</sup>. This transcription unit shows remarkable similarity to the mini-exon repeat at four critical positions (Fig. 6), the remainder of the two sequences sharing no significant homologies. First, the snR3 gene also has the octanucleotide TATTTTGTG centred at position -27 relative to the start site for transcription. Second, the



**Fig. 6** Nucleotide sequence of the mini-exon repeat of *T. brucei* compared with that of the small nuclear RNA (snR3) gene of yeast (from ref. 32). The two sequences are aligned at the indicated positions with position +1 in both cases corresponding to the 5'-terminus of their respective transcripts. Question marks designate functions which have not yet been confirmed.

initiation site for transcription lies within a heptanucleotide sequence which is very similar in the two genes (six out of seven bases identical). Third, both sequences, although of different overall length (137 versus 194 nucleotides for medRNA and snR3, respectively), end near the beginning of an extremely T-rich region. Finally, the region containing the putative donor splice site of the medRNA also shares a 5- out of 6-residue homology with the yeast snR3, the potential significance of which is discussed below.

### Role of medRNA in VSG gene expression

The finding of medRNA fulfils one of the predictions of a model for VSG gene expression involving discontinuous transcription. However, it is not definitive because of the multiplicity of the mini-exon repeats: it could be that, although the majority of the repeats are indeed transcribed to give the short medRNA, one repeat is used in the initiation of a very long transcript including the VSG coding exon. This is a difficult possibility to exclude because pulse-chase experiments, which have been traditionally used to demonstrate precursor/product relationships, suffer from the same shortcoming—it is impossible to demonstrate that a given mini-exon repeat is used for one purpose compared with another, indistinguishable repeat. Nevertheless, together with published observations regarding the abundance of transcripts containing mini-exon-derived sequences and the inability to detect linkage between a mini-exon sequence and the VSG coding exon, the identification of a short, discrete medRNA strongly supports a model of discontinuous transcription. In this model, the mini-exon-derived leader segment of VSG

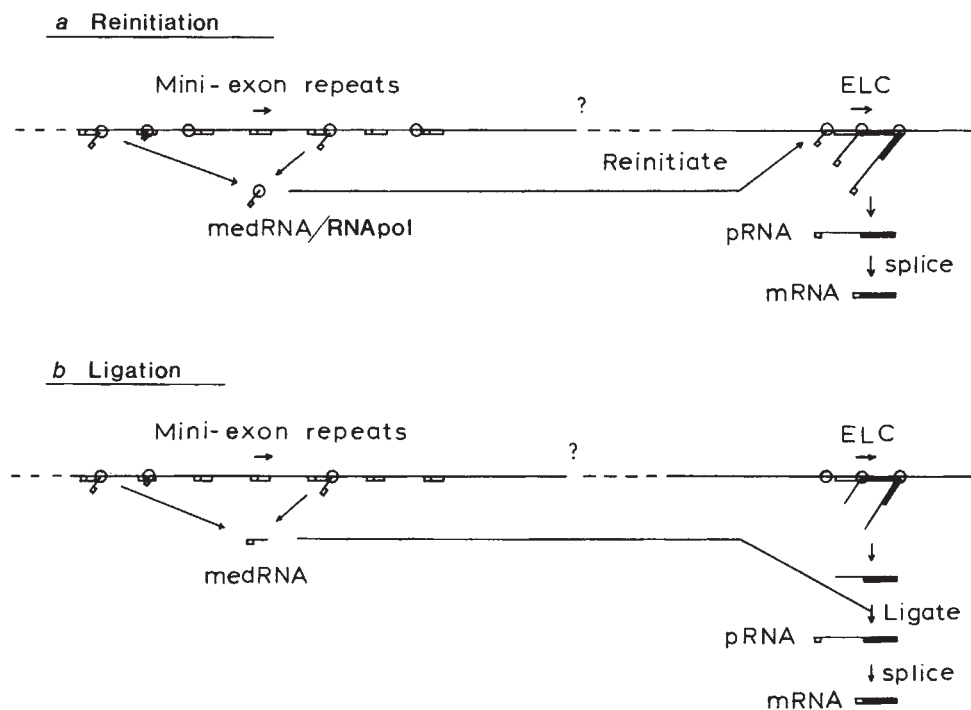
mRNAs is transcribed as a discrete product of the mini-exon repeats and, by one of several possible mechanisms, is ultimately found attached to the 5'-end of the VSG precursor RNA. The chimaeric intervening sequence is then removed by splicing, bringing together the 35-nucleotide leader with the coding portion to give the final mRNA of ~1.7 kb.

There are two general schemes by which this can most easily be imagined to occur. The first model, presented in Fig. 7a, predicts that RNA polymerase transcribes the medRNA and then dissociates from the mini-exon repeat, possibly as a medRNA/polymerase complex; transcription then reinitiates just upstream of the ELC using medRNA as a primer. The second model (Fig. 7b) proposed that medRNA and the coding-region RNA are independently produced as discrete transcripts and then post-transcriptionally ligated to each other. In both cases, splicing ultimately removes the intervening sequence to generate the mature mRNA.

The critical difference between the two alternatives is that post-transcriptional ligation requires a functional promoter (in the sense of a site for *de novo* initiation of transcription) upstream of the ELC whereas the reinitiation model proposes a site in this same position where RNA polymerase could only act in the presence of a primer (that is, medRNA). We are now attempting to test for this distinction *in vitro*.

We have previously reported the sequence upstream of an expressed copy of a VSG gene and compared it with a silent copy of that gene<sup>19</sup>. We found that the upstream region of the expressed copy consisted of multiple tandem repeats of ~76 bp flanking an unusual sequence of (TAA)<sub>90</sub>. Although three 76-bp repeats are found upstream of the silent copy, (TAA)<sub>90</sub> is unique to the expressed copy of this gene and is, therefore, a candidate for the (re)initiation site upstream of expressed VSG genes. If this sequence is unique and mobile, it could be the basis for the mutual exclusion observed between the different expression sites. Equally, another, as yet undetected, mobile control element might have this role.

A critical question raised by the results reported here is whether discontinuous transcription involving a small RNA is a unique property of *T. brucei*. Is it, for example, a special adaptation which has co-evolved with antigenic variation? This is argued against by the finding that the same or similar mini-exon sequences are found in many RNAs (of unknown coding function), not only from bloodstream-forms of the parasite, but also from the procyclic insect forms where VSG genes are not



**Fig. 7** Two models for discontinuous transcription of VSG mRNAs. *a*, The reinitiation model; *b*, the ligation model. The figure illustrates schematically the important features of the two models which are described in the text. The region of the mini-exon repeat which codes for the medRNA is represented by an open segmented box, the left half indicating the mini-exon coding for the 35-nucleotide spliced leader. The ELC region is also represented as a segmented box with the protein-coding exon shaded. The exon-derived portions of the precursor RNA (pRNA) are boxed in the same way. Uncertainty about whether the mini-exon repeats are linked to the ELC and if so over what distance, is indicated by a question mark over the dashed region. RNA polymerase is represented by an open circle.



expressed (ref. 21 and M. Parsons *et al.*, personal communication). In addition, the mini-exon sequence (or a conserved homologue) has been shown to be present in the genomes of several related species and genera which lack antigenic variation of the type observed for *T. brucei*<sup>22</sup>.

Could discontinuous transcription be operating in these other cases? This question cannot, as yet, be answered, but some precedent does exist in systems which are otherwise totally unrelated. Transcription of the influenza virus is known to require a 5'-capped primer (10–15 nucleotides long) derived from cleavage of host mRNAs<sup>36,37</sup>. Coronavirus transcripts, on the other hand, are known to have a common 5'-leader sequence which is seemingly not joined to the coding portion of the RNA by conventional splicing, again strongly suggesting discontinuous transcription<sup>38,39</sup>. The fact that, in both these cases, transcription occurs by an RNA-dependent viral transcriptase may be an important distinction but they do provide a mechanistic precedent for discontinuity in primary transcripts. The

observation made in Fig. 6 that a snRNA of yeast has substantial homology with the region known to be a donor splice site in medRNA, suggests that this snRNA (of unknown function) might be similarly involved in discontinuous transcription, albeit on a lesser scale (snR3 is nonessential; ref. 32). Such activity could further provide a molecular basis for the long-standing observation that heterogeneous nuclear RNAs often possess sequences at their 5'-ends which are derived from middle repetitive DNA<sup>40–42</sup> and that such sequences might be important in the control of developmentally regulated gene expression. The testing of this interesting hypothesis in trypanosomes may now be possible.

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## LETTERS TO NATURE

### On the origin of Triton and Pluto

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Lyttleton hypothesized long ago that Triton and Pluto originated as adjacent prograde satellites of Neptune<sup>1</sup>. With the presently accepted masses of Triton and Pluto–Charon<sup>2,3</sup>, however, the momentum and energy exchange that would be required to set Triton on a retrograde trajectory is impossible. The mass of Triton has probably been seriously overestimated<sup>4,5</sup>, but not by enough to relax this restriction. It is implausible that the present angular momentum state of Pluto–Charon has been significantly influenced by Neptune<sup>6</sup>. It could not acquire such angular momentum during an ejection event unless a physical collision was involved, which is quite unlikely. The simplest hypothesis is that Triton and Pluto are independent representatives of large outer Solar System planetesimals. Triton is simply captured, with potentially spectacular consequences that include runaway melting of interior ices and release to the surface of clathrated CH<sub>4</sub>, CO and N<sub>2</sub> (ref. 7). Condensed remnants of this proto-atmosphere could account for features in Triton's unique spectrum<sup>8–11</sup>.

The dynamics of Triton's orbital evolution are considerably simplified by the fact that its specific dissipation function,  $Q$ ,

at tidal frequencies, is much less than that of Neptune ( $Q_T \ll Q_N$ ). Here I assume a standard solid-body  $Q$  for Triton of  $\sim 100$  (ref. 12). A lower bound on  $Q_N$  can be derived by requiring that the outward orbital evolution of a satellite given by

$$\frac{da_s}{dt} = 3k_{2N} \sqrt{\frac{G}{m_N}} \frac{R_s^5 m_s}{Q_N a_s^{11/2}} \quad (1)$$

is not so rapid that the satellite originated at the corotation radius of Neptune 4,500 Myr ago<sup>12</sup> (where  $m_s$  and  $a_s$  are the satellite's mass and semimajor axis;  $m_N$ ,  $R_N$  and  $k_{2N}$  are Neptune's mass, radius, and tidal-effective potential Love number of the second degree; and  $G$  is the gravitational constant).  $k_{2N}$  is estimated at 0.43, subject to uncertainties in Neptune's rotation rate and  $J_2$  (the coefficient of the second harmonic of the gravitational potential) (see ref. 13); other parameter values are given in Table 1. If Neptune's third satellite<sup>14</sup> is confirmed and proves to be regular and non-commensurate, then  $Q_N \geq 10^4$ . A lower bound on the  $Q$  of Uranus of  $\sim 2 \times 10^4$  is set using Miranda<sup>15</sup> and a  $k_2$  for Uranus of 0.28. The  $Q$ s of both planets should be comparable, and are probably much larger.

Accordingly, the monthly radial tide raised on Triton by Neptune dominates Triton's orbital evolution, except for orbits of very small eccentricity. This tide does not transfer angular momentum for a synchronously-rotating Triton, but in the cases of interest here, such non-synchronous spin angular momentum would be negligible compared with orbital angular momentum. The present fractional rate of change in Triton's orbital angular momentum, due to the tide raised on Neptune by Triton, is

$(da_T/dt)2a_T$ , where  $a_T$  is Triton's semimajor axis. Using equation (1), the total change over the age of the Solar System is a few per cent for  $Q_N \sim 10^5$ . Changes in inclination are similarly small.

Lyttleton's hypothesis is evaluated as follows: Triton and Pluto originate as regular direct satellites of Neptune, in circular orbits; orbital evolution brings them into a range of significant gravitational interaction, say, within the sphere of influence of the more massive satellite (the radius of which is given by  $s = a_>(m_>/m_N)^{2/5}$  (ref. 16), where  $a_>$  and  $m_>$  are the semimajor axis and mass of the more massive satellite), and Triton is sent into a retrograde orbit, potentially elliptical, with the same angular momentum as it has today. Conservation of angular momentum requires that the angular momentum of Pluto,  $l_P$ , increases to

$$l_P = (Gm_N)^{1/2} \{ (\alpha m_T \sqrt{a_T} + m_T \sqrt{a} + m_P \sqrt{a})^2 + \beta^2 m_T^2 a_T \}^{1/2} \quad (2)$$

where  $m_T$  and  $m_P$  are the masses of Triton and Pluto-Charon,  $\alpha$  and  $\beta$  are  $\cos(21.5^\circ)$  and  $\sin(21.5^\circ)$ —accounting for Triton's inclination of  $158.5^\circ$  (ref. 17), and  $a$  is the semimajor axis of the interaction. Pluto's post-encounter orbital energy is minimized for velocities perpendicular to its radius vector. The semimajor axis (elliptic or hyperbolic) of the new orbit, with respect to Neptune, is then:

$$a_P = [l_P^2 / Gm_N m_P^2 a - 2]^{-1} a \quad (3)$$

If  $l_P > (2Gm_N a)^{1/2} m_P$ , the orbit will be hyperbolic. This is guaranteed for  $m_T > (\sqrt{2} - 1)m_P$ .

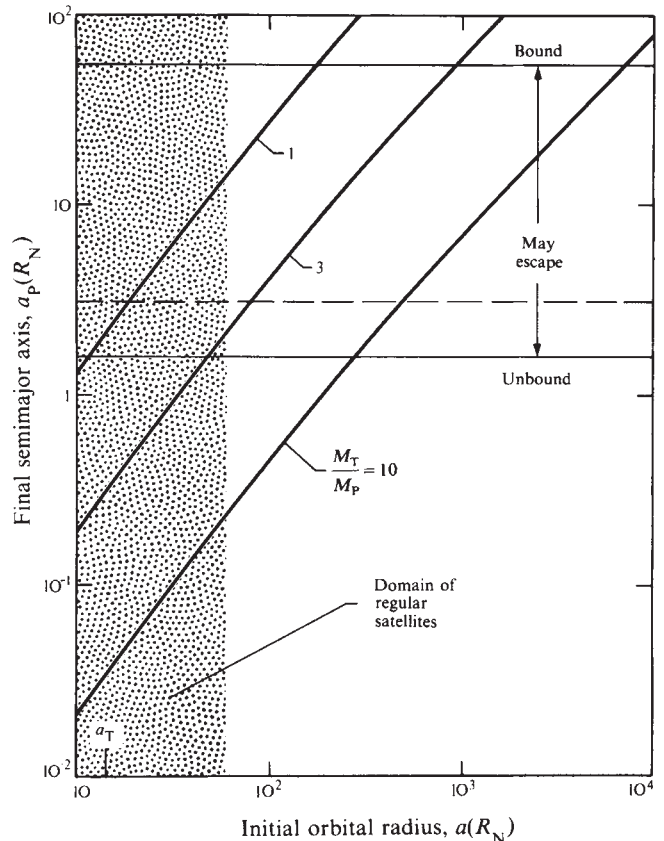
The relationship (3) is illustrated in Fig. 1 for a range of plausible mass ratios  $m_T/m_P$ . All the  $a_P$  shown correspond to hyperbolic trajectories. Orbits with  $a_P < 1.62 R_N$ , however, are so energetic that once Pluto leaves Neptune's sphere of influence, it is not bound to the Solar System. Only for  $a_P > 54.9 R_N$  is Pluto definitely bound. The accepted mass of Triton,  $1.32 \times 10^{26}$  g, is due to Alden<sup>2</sup>. The combined mass of Pluto and Charon is known more precisely ( $\pm 5\%$ ) (ref. 3) and suggests  $m_T/m_P \sim 10$ . In this case, momentum conservation alone determines that a Lyttleton-type interaction is not possible, unless the interaction takes place well outside the domain of regular satellites. This domain,  $a \leq 60 R_N$ , is defined in analogy with the regular satellite systems of Jupiter, Saturn and Uranus. The outermost regular satellite in each system, Callisto, Iapetus and Oberon, orbits at 27, 59 and 23 units of its respective planetary radius.

Recent radiometric measurements<sup>4,18,19</sup> call Triton's mass estimate into question. Lebofsky's  $2\sigma$  detection, in particular, yields a radius of  $1,750 \pm 250$  km (ref. 19). In lieu of accepting Triton as a heavy metal satellite, I assume a density appropriate to an ice-rock body of this size ( $\sim 1.6 \text{ cm}^{-3}$ ) (ref. 20)—implying that  $m_T/m_P \sim 3$ . This lower mass ratio, however, does not make Lyttleton's hypothesis more physically acceptable. Note that neither the effect of a non-unity emissivity nor a surrounding atmosphere has been included in the radiometric modelling, and these would have competing effects on the derived radius. A better determination of Triton's mass is needed.

These arguments can be generalized to allow multiple encounters if the conservation of energy is accounted for. The encounters are assumed to be conservative, with the only restriction on Triton's final energy,  $-Gm_N m_T / 2\tilde{a}_T$ , where  $\tilde{a}_T$  is Triton's final semimajor axis, being that it exceed Triton's present value. The horizontal curves in Fig. 2 are Pluto's final semilatus rectum,  $p_P = l_P^2 / Gm_N m_P^2 = a_P [1 - e_P^2]$ , where  $e_P$  is Pluto's final eccentricity, for various  $m_T/m_P$ . The vertical curves are Pluto's final semimajor axis, given by

$$a_P = a \left\{ \frac{m_T}{m_P} \left( \frac{a}{\tilde{a}_T} - 1 \right) - 1 \right\}^{-1} \quad (4)$$

for  $m_T/m_P = 10$  and two representative values of  $\tilde{a}_T$ . Ascending asymptotes (with increasing  $a$ ) correspond to bound orbits. For ellipses, however,  $p < a$ , so only those  $a_P \geq p_P$  correspond to possible orbits. In addition, the pericentre distance,  $p_P/(1 + e_P)$ ,



**Fig. 1** Hyperbolic semimajor axis of Pluto, with respect to Neptune and after an interaction with Triton, as a function of initial orbital radius for three different mass ratios. These results, derived from angular momentum conservation alone, represent maximum values. Asymptotic velocities from Neptune of 2.25 and  $13.1 \text{ km s}^{-1}$  ( $a_P = 54.9$  and  $1.62 R_N$ ) correspond to limits on bound or unbound solar trajectories, if Neptune's orbital velocity is taken into account. The dashed horizontal line is the minimum  $a_P$  for ejection into a direct, bound solar orbit. For plausible mass ratios, any interaction to reverse Triton's orbital motion and retain Pluto in the Solar System must take place well outside the domain of regular satellites (see text).

has a minimum of  $p_P/2$  for  $e_P = 1$ , and Triton's apocentre distance has a maximum of  $\sim 2\tilde{a}_T$ . Thus even for  $m_T/m_P = 1$ , no bound orbits are possible because the final orbits of Triton and Pluto must be  $\sim$ intersecting ones. A descending branch of  $a_P$ , for a given  $\tilde{a}_T$ , is the locus of unbound states (with respect to Neptune). The top part of the descending branch ( $a_P \geq p_P$ ) corresponds to eccentricities of between  $\sqrt{2}$  and 1; hence these orbits are also non-intersecting and are ruled out. The bottom of the descending branch corresponds to hyperbolic orbits with  $e_P > \sqrt{2}$ ; hence, for large enough  $e_P$ , a pericentre distance small enough to allow intersecting orbits may appear feasible. The situation is very restricted, however, as  $a_P$  has a horizontal asymptote at  $\tilde{a}_T m_P / m_T$ . This limits  $e_P$  to  $\{p_P/a_P + 1\}^{1/2}$ . Pluto's final pericentre distance remains  $> 2\tilde{a}_T$  for  $m_T/m_P \geq 1$ , regardless of the choice of  $\tilde{a}_T$  or initial  $a$ . Thus, for plausible mass ratios, and under the stipulated initial conditions, a combination of orbital interactions to reverse Triton's motion cannot be found.

This conclusion, more severe than that due to momentum conservation alone, is not changed by considering tidal interaction with Neptune during the encounters. In addition, allowing the initial conditions to be set when the difference between the semimajor axes of the two 'satellites' closes to within the radius of either the sphere of influence,  $s$ , or an even wider sphere of perturbation, can bring Triton and Pluto's final orbits closer together (if Triton is the innermost of the pair), but not



**Table 1** Parameter values

| Object  | Quantity        | Value                                        | Reference                                                          |
|---------|-----------------|----------------------------------------------|--------------------------------------------------------------------|
| Neptune | $m_N$           | $1.03 \times 10^{26}$ kg                     | 36                                                                 |
|         | $R_N$           | $24.75 \times 10^3$ km;<br>1 bar, equatorial | 37                                                                 |
|         | $J_2$           | $4.3 \times 10^{-3}$                         | 17                                                                 |
|         | Rotation period | $\sim 15$ h; dynamical                       | 38                                                                 |
| Uranus  | Mass            | $8.67 \times 10^{25}$ kg                     | 36                                                                 |
|         | Radius          | $25.5 \times 10^3$ km; 1 bar, equatorial     | 39, 40                                                             |
|         | $J_2$           | $3.35 \times 10^{-3}$                        | 40                                                                 |
|         | Rotation period | 15.5 h; dynamical                            | 40                                                                 |
| Triton  | $a_T$           | $354.3 \times 10^3$ km                       | 17                                                                 |
| Pluto   | $R_P$           | $\sim 1,500$ km                              | 41                                                                 |
|         | $Q_P$           | $\sim 100$                                   | Assumed<br>Based on unity<br>cgs density and<br>water-ice rigidity |
|         | $k_{2P}$        | $\sim 2.7 \times 10^{-2}$                    |                                                                    |
|         |                 |                                              |                                                                    |

sufficiently to have them interacting for  $m_T/m_P \geq 1$ . Of course, for  $m_T \ll m_P$ , Triton can be sent retrograde, but Pluto will remain direct.

The present angular momentum state of the Pluto–Charon system (that is, that contained in their mutual orbits and spins) is not consistent with a Neptune satellite origin either. If Pluto–Charon ever orbited Neptune with a semimajor axis  $a$ , tides raised on Pluto by Neptune would extract angular momentum from the system at a rate

$$\frac{dH}{dt} = \frac{3}{2} \frac{Gk_{2P}m_N^2R_P^2}{a^6Q_P} \quad (5)$$

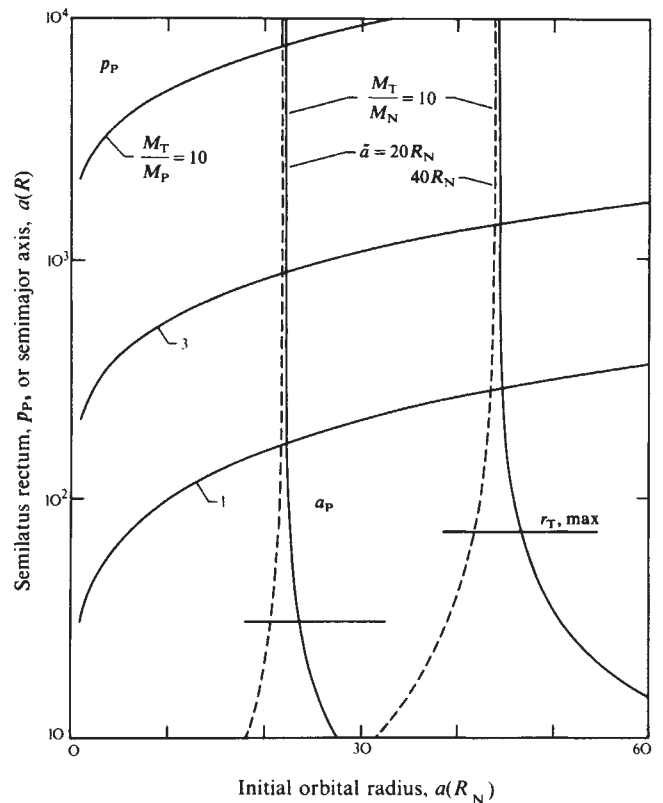
where  $H$  is the angular momentum, and  $R_P$ ,  $Q_P$  and  $k_{2P}$  are, respectively, Pluto's radius, specific dissipation function and potential Love number (see Table 1). The present angular momentum of Pluto–Charon is uncertain due to the difficulty in estimating Charon's mass, but probably lies between 2.4 and  $6.6 \times 10^{37}$  g cm<sup>2</sup> s<sup>-1</sup>. Extraction of angular momentum would cause the Pluto–Charon system to contract and eventually coalesce. The coalesced object would then be spun down to synchronous rotation<sup>6</sup>. The time scale for 'fusion' and spin-down is

$$\tau = H / \left( \frac{dH}{dt} \right) \sim 1 \times 10^5 \left( \frac{a}{a_T} \right)^6 \text{ yr} \quad (6)$$

Thus collapse of the Pluto–Charon system and spin-down would occur rapidly unless Pluto–Charon orbited Neptune at a great distance ( $\tau \geq 100$  Myr for  $a > 45R_N$ ). These same arguments apply if the original state of Pluto–Charon was a coalesced, rapidly-rotating proto-Pluto (the angular momentum limits above correspond to primordial rotation periods between  $\sim 7$  and 11 h; see refs 21, 22).

Only a physical collision could impart the requisite angular momentum to Pluto–Charon prior to its ejection from the Neptune system. A close pass to Pluto by a more massive object could exert an extremely large torque on Pluto but the time scale for the interaction would be severely limited. A grazing pass by an object with even the mass of the Earth would be restricted to last a few  $10^3$  s, and the angular momentum imparted, according to equation (5), would be only a few per cent of the present total. A larger transfer of angular momentum may be possible for the slowest encounters with a massive grazer, if tidally-induced fission<sup>23</sup> occurs, but the restriction of slow encounters again requires Pluto to initially be in a distant orbit about Neptune.

With regard to ejection dynamics, the reduction of the interaction cross-section from a gravitational one to approximately one of collision makes 'rogue' planet hypotheses such as that of



**Fig. 2** Semilatus rectum and semimajor axis of Pluto, with respect to Neptune and after interactions with Triton, as a function of initial orbital radius for various mass ratios and final Triton semimajor axes. Only the solid part of the asymptotes represent possible orbits, but none have pericentre distances that approach Triton's final apocentre distance (short horizontal lines). Hence, for mass ratios greater than one, a series of interactions that reverses Triton's orbital motion and that conserve angular momentum and orbital energy cannot be found.

Harrington and Van Flandern<sup>24</sup> probabilistically untenable. Farinella *et al.* call on a captured Triton to eject Pluto as its capture orbit decays<sup>25</sup>, but this hypothesis still requires a physical collision with Pluto, following which, gravitationally-bound plutonian debris accretes into a binary and eventually reaches stable, resonant<sup>26</sup> solar orbit; the last requires an additional mechanism. This is an unlikely series of events.

The preceding arguments effectively decouple the origin of Pluto from that of any Neptune satellite. Hence, for Triton to begin as a direct satellite and end up retrograde would require interaction with an unknown and *ad hoc* object. I propose a simple reversal of Lyttleton's hypothesis: instead of beginning as satellites of Neptune, Triton and Pluto originate as satellites of the Sun. The existence of large, outer Solar System planetesimals is consistent with the dynamics of planetary accretion and may even be necessary to explain planetary eccentricities, inclinations, and obliquities (see ref. 27). Pluto–Charon's binary status could be related to hypotheses regarding binary asteroid formation,<sup>21,28–30</sup> and Triton's capture could be effected by the combination of a retrograde, temporary orbit about Neptune<sup>31</sup> and a dissipative mechanism such as gas drag<sup>32,33</sup>.

If Triton had been captured, then it should have experienced a spectacular thermal event. As  $Q_N \gg Q_T$ , nearly all of the orbital energy dissipated during post-capture orbital evolution is deposited in the body of Triton. Gas drag, while potentially important for capture, did not result in a major change in orbital elements, as the evolution of Triton's inclination has been moderate<sup>32</sup>. Calculations<sup>7</sup> show that collapse of an elliptical post-capture orbit, extending to the edge of Neptune's sphere of influence ( $\sim 3,500 R_N$ ), to one where the semimajor axis is

within a few per cent of the present value, requires  $\sim 4 Q_T$  Myr, for a constant  $Q_T$ . Most of the energy ( $\sim 80\%$ ), however, is dissipated in an  $\sim 1.5 Q_T$  Myr period near the end. The equivalent surface heat flow, for reasonable  $Q_T$ , is far too great for solid-state convection in ice to transport, so it is extremely probable that the icy component of Triton completely melted during the latter phase of capture.  $Q_T$  itself would plummet as melting began, accelerating differentiation.

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Catastrophic melting of Triton's ices should have released its inventory of clathrated gases to the surface, which for accretion outside a circumplanetary nebula would be  $\text{CH}_4$ ,  $\text{CO}$ , and  $\text{N}_2$  (refs 34, 35). A condensed fraction of this primordial atmosphere may be responsible for Triton's unique surface composition—present spectral identifications include liquid nitrogen<sup>11</sup> and methane ice<sup>9–11</sup>.

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## X-ray haloes around supernova remnants

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Recent observations of the Cas-A supernova remnant have shown X-ray emissions not only from the interior, but also from a fainter 'halo' extending beyond what is normally regarded as the outer boundary, or shock front<sup>1</sup>. We suggest here that this may be due to the diffusion of energetic, charged particles out of the remnant giving rise to precursor structure of the type predicted by the theory of diffusive shock acceleration. If this is the case we are seeing thermal emission from ambient gas heated by compression and wave dissipation.

The radio synchrotron emission from supernova remnants is clear evidence that they contain relativistic electrons; it is widely believed that they are also the major source for the galactic cosmic-ray nucleon component, but so far there has been no direct observational evidence for this. Cosmic-ray nuclei have an energy density which exceeds that of the electrons by about two orders of magnitude; for supernova remnants to be responsible for their origin, this implies that a substantial part of the remnant energy must reside in energetic charged particles<sup>2</sup>. This raises the question of how these particles are accelerated. Interstellar plasmas are typically so tenuous that cosmic rays are scattered only by the irregular structure of the magnetic field. Because the field lines are effectively frozen into the background plasma these irregularities can move relative to the bulk fluid at maximum speeds of the order of the magnetosonic speed. As Fermi<sup>3</sup> recognized, this is a rather unusual physical system in that macroscopic degrees of freedom, the magnetic fluctuations and their associated plasma, couple to a few individual particles. If the fluctuations are excited randomly this leads to a diffusion of the particles in momentum space which is the standard second-order Fermi acceleration mechanism. This is normally slow; however, at a shock front where the bulk plasma together with its embedded magnetic structures is suddenly decelerated (in the shock frame) the scatterers, instead of moving randomly,

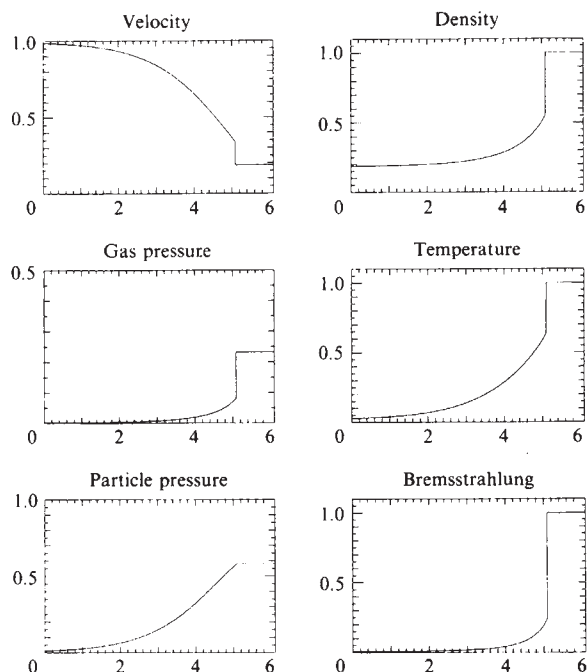
converge, giving rise to a much faster first-order acceleration<sup>4–8</sup>. Perhaps the major attraction of this process, usually called diffusive shock acceleration<sup>9–11</sup>, is that an elementary analysis in which the reaction of the particles is neglected, predicts that the accelerated particles should have power-law spectra in momentum with exponents close to those inferred for the sources of the galactic cosmic rays.

It is, however, clear that the reaction of the particles on the scattering irregularities, and through these on the bulk plasma flow, cannot be ignored if the energy density of the particles is significant, as is thought to be the case in supernova remnants<sup>5,12</sup>. The particles diffuse ahead of the shock into the upstream region and produce an adverse pressure gradient which decelerates the flow into the shock. This gradient also works on the scatterers at the rate  $-v \cdot \text{grad } P_c$ , where  $v$  is the velocity of the scatterers and  $P_c$  is the energetic particle pressure; clearly, and as one would intuitively expect, those fluctuation modes which move upstream relative to the plasma are amplified and those which move downstream are damped. The energy transferred to the amplified modes can be estimated to be of the order of the Alfvén Mach number of the shock times the background magnetic field energy<sup>9,13</sup>. For strong astrophysical shocks, Alfvén Mach numbers of 20 or greater are expected. Thus in the absence of any dissipation, or radical change in the nature of the modes, the energy content of the fluctuations would become many times that of the background field; it seems doubtful that so much energy could be stored without various instabilities setting in which transfer excess energy to the plasma and keep the fluctuation amplitude of the order of unity.

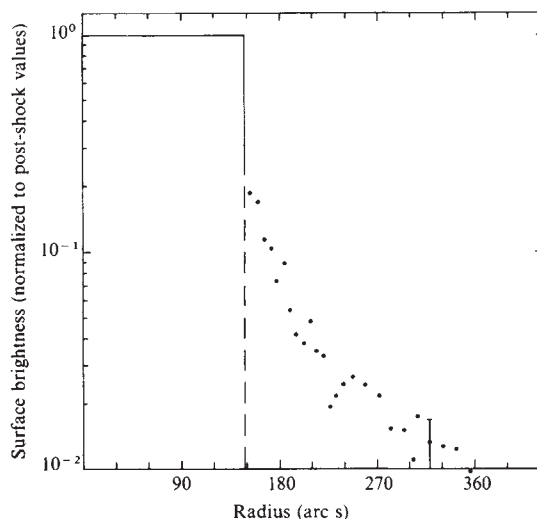
Although the details of this process are very complicated, its main features have been described by a simple model<sup>13–15</sup> in which only the energy and momentum fluxes associated with the thermal particles (treated as an ideal non-relativistic gas), the energetic particles (treated as an ideal relativistic gas) and the magnetic fluctuations (taken to be damped backwards-travelling Alfvén waves) are considered, while the shock structure is taken to be plane and steady.

We have calculated the shock structure and the Bremsstrahlung emissivity predicted by this model. Figure 1 shows an example of a shock with Alfvénic Mach number 20 and equal thermal particle, energetic particle and magnetic pressures far upstream. This high Mach number should be characteristic of young supernova remnant shocks. As can be seen, the shock





**Fig. 1** Effects of cosmic rays on parallel magneto-hydrodynamic shocks. The values of the gas-velocity, gas-pressure and cosmic-ray pressure are normalized such that the total mass and momentum fluxes are unity. All pressures are equal far upstream, and the Alfvén Mach number is 20. The values of the gas-density, gas-temperature and, as a Bremsstrahlung-emissivity measure,  $(\text{density})^2 \times \sqrt{\text{temperature}}$  are normalized to the post-shock region. The horizontal axis gives the length scale in units of diffusion coefficient/shock velocity.



**Fig. 2** Surface brightness of the Cas-A 'halo' plotted as a function of distance from the shock. The 'halo' emission is the excess derived by Stewart *et al.*<sup>1</sup>; the data have been normalized to the remnant brightness of the outer ring, and the detailed internal structure of the remnant has not been considered.

acceleration of cosmic rays (predominantly nuclei) is rather important. The cosmic-ray pressure, in the steady model, is about twice the thermal gas pressure and about twice the kinetic energy density. Also, the gas density and velocity profiles are modified ahead of the shock, and the gas is pre-heated by the Alfvén wave decay and the compression in front of the shock. The 'precursor' length scale is rather uncertain because it depends on the (poorly known) diffusion coefficient  $\kappa$  of the particles; however, if they are accelerated at the shock then  $\kappa \leq R\dot{R}$ , where  $R$  is the shock radius. The 'precursor' length scale is then less than, or comparable to, the shock radius. The point of interest is that wave dissipation and compression together heat the gas in the precursor to temperatures which are not much lower than those behind the shock.

This implies that the processes described above give rise to extended thermal X-ray (Bremsstrahlung and line) and possibly coronal line (for example, [Fe XIV] 5,303 Å and [Fe X] 6,374 Å) emission outside the shock radius of a young supernova remnant. From an observational point of view this extended halo might be difficult to detect because of its low surface brightness (see Fig. 1). Only Cas-A is definitely known to possess an extended halo, from the Einstein Observatory measurements. Several physical processes for this phenomenon have been discussed, such as, electron 'leakage' ahead of the shock, shock heating by ejecta fragments and scattering by interstellar dust<sup>1</sup>. We present here an alternative interpretation, which could be checked, for instance, by a search for haloes around point sources, and other supernova remnants.

Figure 2 shows the radial variation of the X-ray excess surface brightness outside the shock, in the case of Cas-A. The excess emission outside the shock was normalized to the emission just behind the shock, which, ignoring the complicated internal remnant structure, is simply represented as a constant level. A halo length scale of  $\sim 50$  arc s is indicated, roughly one-third of the remnant radius. This is in agreement with the upper bound

determined from the theory, and, therefore, we tentatively suggest that the faint 'haloes' may be produced by the processes described above. This being the case, we are seeing evidence for a dynamic role of cosmic rays in the interstellar medium, and for the acceleration of nuclei in supernova remnants. A comparison with radio observations of the synchrotron emission may then allow a determination of the relative intensities of the nucleonic and electronic components. If confirmed by further observations, this will have important consequences for the evolution of supernova remnants<sup>16</sup> and the structure of the interstellar medium. Although the model calculations shown in Fig. 1 are based on diffusive shock acceleration at the outer boundary of the remnant, a similar situation will be produced by an efficient acceleration mechanism inside the remnant, provided that the accelerated particles diffuse out (ref. 1 and H. J. Völk, personal communication). In the case of Cas-A, diffusive acceleration at the outer shock seems to work well; however, in view of the complicated and violent internal structure and abnormally high radio luminosity of Cas-A this may not be the only source of cosmic-ray acceleration. To test our interpretation of the X-ray data, and the implied consequences for the interstellar medium, observations in other spectral regions and very deep X-ray exposures are needed.

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## Spectral feature of 31 December 1981 $\gamma$ -ray burst not confirmed

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The emission lines reported at 400–500 keV in the spectra of  $\gamma$ -ray bursts<sup>1</sup> have been interpreted as redshifted positron annihilation lines, and, together with the lower-energy cyclotron lines<sup>1</sup>, are the most direct evidence that bursts occur on the surface of neutron stars<sup>2</sup>. Therefore, it is important to confirm the existence and determine the properties of these features. So far, the most significant detections of emission features have been reported from the Konus experiments on Veneras 11–14. Possible confirmatory observations have suffered from limited statistical significance or difficulties in interpretation. Here we compare measurements of a  $\gamma$ -ray burst at 01:37 UT on 31 December 1981, using the Solar Maximum Mission (SMM)  $\gamma$ -ray spectrometer with those made by the Konus instruments. The SMM spectra exhibit no evidence for the presence of emission features reported by the Konus group<sup>3</sup>.

Annihilation lines have been reported in ~5% of the bursts observed by the Konus instruments from 1978 to 1982<sup>1,3</sup>. The typical emission features observed by Konus are centred at 400–450 keV and have widths of ~200 keV (FWHM). The strong feature in the 5 March 1979 burst<sup>2</sup> fits this description; however, that event is so unusual that we are reluctant to include it in the same class as other bursts<sup>4</sup>. There are two other Konus line detections for which simultaneous observations were made with other spectrometers. These bursts occurred on 4 and 19 November 1978. The 4 November line had a width ~200 keV, which is typical of the Konus detections, while the 19 November feature was very broad. Barat<sup>5</sup> confirms the presence of both of these lines. Teegarden and Cline<sup>6</sup> also reported a narrow line at ~420 keV in the spectrum of the 19 November burst, using data from the ISEE 3 germanium spectrometer, but the statistical significance was marginal at best. They found no evidence for a line in the 4 November burst. Fenimore *et al.*<sup>7</sup> analysed the 4 November burst using the scintillation spectrometer on ISEE 3; they found that the apparent strength of the ~400-keV line was strongly dependent on the choice of continuum model, and could be consistent with zero. They also found no evidence for a line in the 19 November burst.

The present observation was made using the Gamma-Ray Spectrometer<sup>8</sup> (GRS) on the SMM spacecraft. The GRS main detector consists of seven NaI scintillators viewing about half of the sky, centred on the Sun. It covers the energy range 0.3–9 MeV with a resolution of 7% FWHM at 0.662 MeV. The gains of the seven crystals are continuously stabilized by <sup>60</sup>Co calibration sources. For a burst in the centre of the field-of-view, the effective area is 4–6 times larger than that provided by the Konus system at 0.5 MeV. The time resolution for spectroscopy is 16.384 s. We analyse spectra using a detector response function based on calibrations made before and during flight and on a Monte Carlo computer simulation. The line profiles predicted by this response function have been verified in flight by observation of lines from solar flares and the Earth's atmosphere, including the annihilation line at 0.511 MeV (refs 9, 10).

Figure 1 shows the time profile of the burst, as observed by the GRS auxiliary X-ray detector and main detector. The time resolution for spectroscopic observations is illustrated by the 0.35–0.8 MeV profile. This was an unusually long burst (about

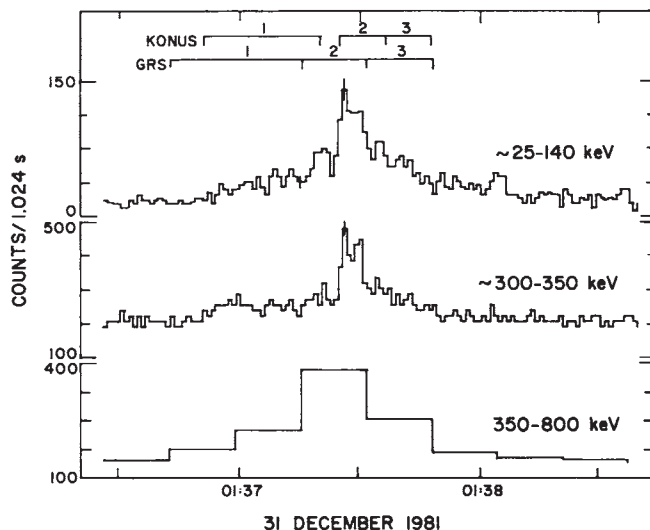


Fig. 1 Time profile of the burst in three GRS energy bands. The top shows the time intervals for which spectra are available from the GRS and Konus instruments.

2 min) with several brief subbursts. The time delay between the GRS and Konus detections (about 53 s) was determined by matching the most prominent of these peaks. The report of the Konus observations<sup>3</sup> includes spectra of three time intervals; these are shown at the top of Fig. 1. Broad emission features extending to high energy were reported for intervals 2 and 3. Also shown are the corresponding intervals for which GRS spectra are available. Because the Konus intervals are long (12 s), a direct comparison is possible.

Based on preliminary data from other satellites and from analysis of the response of the GRS shield detectors, we estimate that the position of the burst is ~50° from the GRS viewing direction. This is well within the GRS field of view. As compared with the on-axis response function, the effective detector area at 50° is reduced by about 15% and a small energy-dependent correction is required because of partial screening by the shield and other parts of the instrument. The effect of this correction is to increase the inferred flux near 500 keV by ~5% with respect to higher and lower energies. Thus, applying this correction will enhance any emission features which may be present. The correctness of this procedure will be demonstrated below by the good agreement of the GRS and Konus results in interval 1.

The Konus and GRS spectra for the three intervals are plotted in Fig. 2. The GRS energy channels have been combined to match the Konus channels. For illustrative purposes, the normalizations of the Konus spectra have been adjusted to compensate for the slightly different accumulation intervals of the two instruments. To accomplish this, we compared the average counting rates in the GRS and Konus time intervals, using the 40–140 keV band of the GRS X-ray detectors (1.024 s time resolution). This adjustment is not used in the quantitative analysis described below. The agreement in absolute flux for the two instruments is good to about 30% in the overlapping energy range (0.3 to ~1 MeV). Of course, these power-law functions could not be extended to lower energy; the gradual curvature of the spectra below ~200 keV is evident in Fig. 2.

A single smooth function could fit the spectra from both instruments in interval 1, with a small shift in relative normalization. However, there is no evidence in the GRS spectra for the features which are found in the Konus spectra in intervals 2 and 3. Power-law spectra will fit the GRS data acceptably over the entire 0.3–0.9 MeV range in all three intervals. Of course, these power-law functions could not be extended to lower energy; the gradual curvature of the spectra below 200 keV is evident in Fig. 2.

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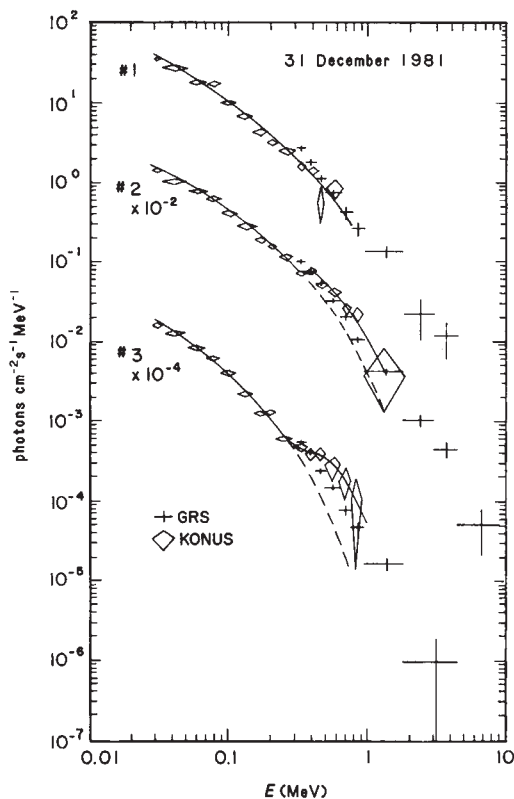


Fig. 2 Photon spectra for the three time intervals from Konus and GRS. The curves represent the models fit to the Konus data by Mazets *et al.*<sup>3</sup>. Solid line, thermal bremsstrahlung + emission feature; dashed line, high-energy extension of bremsstrahlung.

To make direct comparisons between the GRS data and the Konus model spectra, we multiplied the Konus models by the GRS detector response matrix and compared the results with the raw GRS data. We allowed the intensity of the model spectrum to be a free parameter (to account for possible errors in absolute calibration of the instruments), and adjusted it for the best agreement. The comparison was made only in the energy ranges for which both Konus and GRS data are available. In interval 1, the agreement was good:  $\chi^2 = 4.3$  for 3 d.f. The best  $\chi^2$  values obtained for the other two intervals are unacceptable: 138 for 6 d.f. in interval 2, and 137 for 5 d.f. in interval 3. In both cases the model has a flattening in the 0.3–0.5 MeV range, which is not compatible with the GRS data. Figure 3 illustrates the results for interval 3. It shows, in terms of uncorrected detector counts, the GRS data and the Konus model (adjusted in intensity for the best agreement). The value of the upper limit that we can establish on the flux in a broad line depends on the continuum model chosen. Conservatively, our 99% confidence upper limit is 0.5 photons  $\text{cm}^{-2} \text{s}^{-1}$ , about one-quarter of our estimate of the strength of the Konus feature.

We repeated this analysis, omitting the lowest-energy GRS channel group ( $\sim 300$ – $350$  keV). As we have no energy calibration below a 389 keV background line, it is possible that the assumed width of the lowest channel group could be in error. The agreement in interval 1 continued to be good. Even though the lowest channel group contained the greatest number of counts, the best  $\chi^2$  values in the other two intervals remained unacceptable: 44.5 for 5 d.f. in interval 2 and 59.4 for 4 d.f. in interval 3.

The intervals of the two instruments are not identical. However, it is unlikely that a rapid change in the spectrum could have produced the difference in the results. The last two Konus intervals are entirely contained within the combined GRS intervals 2 and 3. The remaining portion of GRS interval 2 is a time of lower burst flux, so its contribution is relatively small. The difference in the results from the two instruments is not subtle.

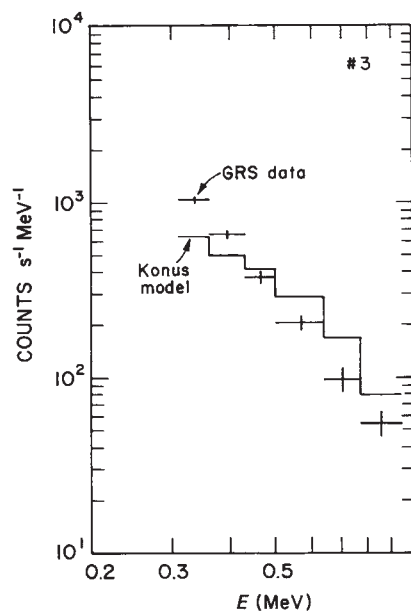


Fig. 3 GRS detector energy loss spectrum of the third interval. The histogram is the Konus model, multiplied by the GRS response matrix, with normalization adjusted for best fit.

There is a flattening of the spectrum in Konus intervals 2 and 3 which is not present in either of the corresponding GRS spectra. In the 0.3–0.5 MeV range, the GRS spectra are fit by power laws with indices of  $-2.00 \pm 0.15$  and  $-2.47 \pm 0.23$ . The two Konus spectra are much flatter, with indices of  $> -1.2$  in this energy range.

It is not certain that we can reconcile the results from the two experiments. As shown above, the continuum shapes are similar, but there are differences in narrow energy bands. We believe that they may be attributed to different data analysis procedures. The Konus data are presented only in the form of 'photon' spectral plots, corrected for the effects of the detector response. The process of producing such graphs is model-dependent, and in many cases, the plotted points tend to move to agree with the model<sup>7</sup>. We do not know to what degree the Konus spectra are prone to such 'compliance', which is due to non-diagonal components in the detector response matrix. The GRS should be less subject to compliance, because the detector is larger and has CsI anticoincidence shields to suppress the detection of Compton-scattered photons. (For example, the derived photon fluxes at 0.3 and 1 MeV vary by only about 20% for a range of input model spectra from  $E^{-1.1}$  to  $E^{-4}$ .) If this effect is not enough to explain the differences, the only other explanation is a very unlikely fluctuation of the spectrum at just the right time. The best understanding of the nature of a spectrum can be obtained from statistical measures of its agreement with various models. However, the statistical significances of the reported features in the Konus data unfortunately are not given.

We have previously reported a weak (0.4 photon  $\text{cm}^{-2}$ ,  $2.5 \sigma$  detection) and relatively narrow feature near 475 keV in the first half of GRS interval 1, which contains about 5 s of the burst<sup>11</sup>. But this is not a significant detection, and it is much weaker than the features reported by Konus later in the burst. Indeed, it is about a factor of 4 weaker than the weakest lines detected by Konus in other bursts. Thus this possible feature can have little to do with the features discussed here. We know of only one other burst observed by GRS (28 March 1982) for which Konus spectra have been published. No annihilation lines were found in either the GRS or Konus spectra in that burst.

The GRS observations are inconsistent with the presence of the reported emission features in the burst of 31 December 1981. Because the existence of features such as these is one of the principal pieces of evidence that bursts occur on neutron stars,

it is important to assess the significance of all the reported Konus line features.

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## Limiting battery performance parameters for polyacetylene

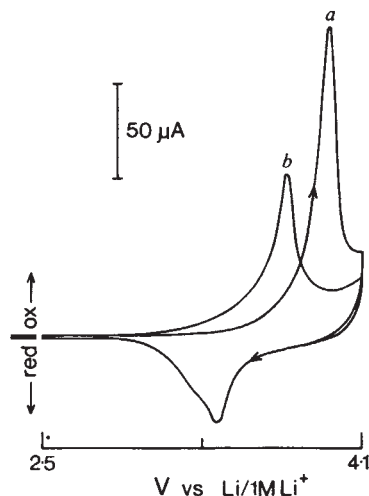
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The discovery that polyacetylene,  $[\text{CH}]_x$ , could be reversibly electrochemically oxidized or reduced ('doped')<sup>1</sup> has led to its usage as an electrode in light-weight rechargeable storage batteries<sup>2,3</sup>. Using a 100- $\mu\text{m}$  thick electrode comprised of 20–30 nm diameter fibrils<sup>4</sup>, performance characteristics slightly superior to those of the lead-acid battery have been achieved. Our cyclic voltammetry study utilizing ultrathin ( $<0.1\ \mu\text{m}$ ) films of  $[\text{CH}]_x$  comprised of 2–3 nm microfibrils shows significantly improved electrode properties. A current density of  $2.4\ \text{MA kg}^{-1}$ , power density of  $8\ \text{MW kg}^{-1}$ , average potential of 3.5 V, maximum energy density of  $250\ \text{Wh kg}^{-1}$  and coulombic efficiency  $>95\%$  have been obtained with a  $\text{Li/salt}/[\text{CH}]_x$  system. The potential of polyacetylene electrodes for light-weight rechargeable batteries is, therefore, several orders of magnitude higher than presently recognized.

Since the minimum detectable amount of  $[\text{CH}]_x$  using cyclic voltammetry is  $\sim 100\ \text{pg}$ , the 20–500 ng of  $[\text{CH}]_x$  deposited on electron microscopy (EM) grids give excellent signal-to-noise ratios, and quantitative calculations involving doping level ( $y$  in  $\text{mol/mol CH}$ ), energy density, efficiency, power density, and current density are possible. These thin films have proved to be ideal for carrying out rapid, numerous electrochemical experiments. The time taken for complete charging and discharging has been accelerated by more than three orders of magnitude. Morphological changes resulting from electrochemical treatment may then be examined directly by electron microscopy and electron diffraction. Because very low currents are involved in our battery experiments, resistive losses in the electrolyte and through the polymer film are negligible, allowing the examination of the limiting charging and discharging characteristics of  $[\text{CH}]_x$  itself.

The method used to polymerize acetylene directly onto EM grids using a Ziegler–Natta catalyst has been described elsewhere<sup>5–7</sup>. A film of  $\sim 0.1\ \mu\text{m}$  thickness was obtained, composed of a network of loosely-packed  $[\text{CH}]_x$  fibrils of 2–3 nm diameter. Platinum wire contact was made to an EM grid and the grid



**Fig. 1** Cyclic voltammetry of 300 ng  $[\text{CH}]_x$  on an EM grid in 1 M  $\text{LiClO}_4$  in propylene carbonate at a  $50\ \text{mV s}^{-1}$  scan rate: *a*, first scan; *b*, second and subsequent scans.

immersed in 2 ml of 1 M  $\text{LiClO}_4$  in dry propylene carbonate, 0.35 M  $\text{LiBF}_4$  in propylene carbonate, or 0.5 M  $\text{LiClO}_4$  in sulpholane in a dry box. Potential and current was controlled with a Princeton Applied Research (PAR) 173 potentiostat, and charge was recorded with a PAR 179 digital coulometer. Potential ramps for cyclic voltammetry were generated using a PAR 175 programmer and fast discharging transients were recorded on a Tektronix 564B storage oscilloscope.

Cyclic voltammetry of *cis*-rich  $[\text{CH}]_x$  on EM grids (Fig. 1) gives clear evidence of electrochemical isomerization to the *trans* polymer, a phenomenon that has been inferred from IR studies on electrochemically-doped  $[\text{CH}]_x$  (ref. 8). The first scan (curve *a*) gives an oxidation peak at  $3.96 \pm 0.05\ \text{V}$  versus  $\text{Li}^+/\text{1 M Li}^+$ . The second and all subsequent scans (curve *b*) give a reproducible peak at  $3.74 \pm 0.03\ \text{V}$ . The fact that complete *cis*–*trans* isomerization occurs on one electrochemical cycle is confirmed by electron diffraction.

Extending the potential range of cyclic voltammetry shows a second, larger peak (Fig. 2c). This oxidation is not reversible and, in fact, extended oxidation of  $[\text{CH}]_x$  leads to the degradation of the electronic properties of doped polyacetylene<sup>9</sup>. Experiments we have done on thicker, previously weighed films show that the stoichiometry of exhaustively oxidized polyacetylene is  $[(\text{CH}(\text{ClO}_4)_{0.5})_x]$ . Thus, we are provided with a direct electrochemical way of determining the total quantity of  $[\text{CH}]_x$  on an EM grid.

The real potential of  $[\text{CH}]_x$  as a battery material lies in very high power density and current density applications. Figure 3 shows the excellent discharge characteristic of  $[\text{CH}(\text{ClO}_4)_{0.06}]_x$  film on an EM grid, discharged at an average power density of  $8\ \text{MW kg}^{-1}$  and a current density of  $2.4\ \text{MA kg}^{-1}$ . These values were obtained at a constant current of 1.0 mA. In the case of the standard thick  $[\text{CH}]_x$ , average power densities of up to  $600\ \text{W kg}^{-1}$  and current densities of  $\sim 20$ – $200\ \text{A kg}^{-1}$  have been reported. This large difference in battery performance parameter is due to the resistive potential losses in film and electrolyte, and limited access of counter ion to  $[\text{CH}]_x$  fibrils. Also, the cyclic voltammetric scan rates of  $2\ \text{mV s}^{-1}$  to  $200\ \text{mV s}^{-1}$  which correspond to charging times of 850 and 8.5 s, respectively, represent an increase of over 1,000-fold in charging rate over that used for thick films<sup>2,3</sup>. Even at these high charging rates, the  $[\text{CH}]_x$  is doped to saturation, indicating that solid-state diffusion of counter ions into  $[\text{CH}]_x$  microfibrils is not a limitation<sup>10</sup>.

Although a maximum reversible dopant level of 6% was achieved by oxidation of the  $\sim 20\ \text{nm}$  fibrils found in thick films<sup>2,3</sup>, it was generally hoped that by varying the morphology of the polymer or the composition of the electrolyte, higher oxidation levels, and hence energy densities, could be obtained<sup>3</sup>.



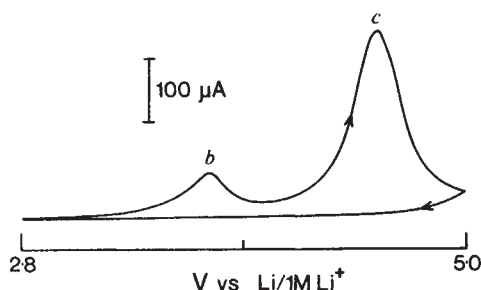


Fig. 2 The exhaustive oxidation of  $[CH]_x$  on an EM grid at  $50 \text{ mV s}^{-1}$  scan rate.

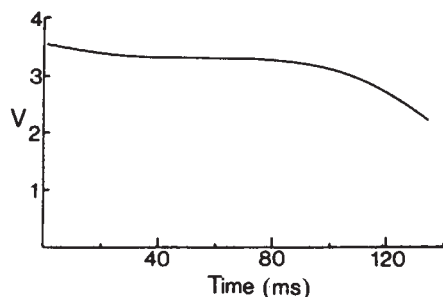


Fig. 3 Fast discharge of a  $[CH(ClO_4)_{0.06}]_x$  sample on an EM grid at  $2.4 \times 10^6 \text{ A kg}^{-1}$ .

For example, if the maximum dopant level were a surface-area related phenomenon, thinner  $[CH]_x$  fibrils should lead to a higher energy density. We have found that the maximum reversible oxidation level for the  $\sim 3 \text{ nm}$  fibril of  $[CH]_x$  on EM grids is  $6 \pm 1\%$  which is relatively insensitive to the counter ion. The average discharge voltage compared with  $Li^0/1 \text{ M Li}^+$  is  $3.4 \text{ V}$  giving an energy density of  $280 \text{ Wh kg}^{-1}$  which falls in the range of  $176\text{--}340 \text{ Wh kg}^{-1}$  found for thick films<sup>3,11</sup>.

Cycling between 2.5 and 4.2 V, the potential at which  $[CH]_x$  starts to degrade, gives coulombic efficiencies in excess of 95% when corrected for background (bare EM grid). This is compared with the 80–90% efficiency for the thick  $[CH]_x$  electrodes. Note that the discharge of polyacetylene cannot be represented as the charging of a capacitor<sup>12</sup>, as the constant current discharge characteristics of a capacitor would be linear and one would expect the energy density ('capacitance') to be proportional to the surface area of the  $[CH]_x$  for ultrathin and thick  $[CH]_x$  films.

This study establishes some of the limiting battery performance parameters for  $[CH]_x$ . Taking average current and power densities obtained with thick  $[CH]_x$  films, one can seriously underestimate the true potential of  $[CH]_x$ . Realization of even fractional gains in these expected current and power densities could improve the technology of light-weight rechargeable batteries.

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## Thermoluminescence as a palaeothermometer

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Chondritic meteorites are widely regarded as a unique source of information on the chemical and physical processes that occurred in the early Solar System. An important phase in their history is a period of metamorphism<sup>1</sup> and thermoluminescence (TL) has proved a powerful technique for exploring this aspect, especially at the low end of the metamorphic spectrum<sup>2</sup>. The ordinary chondrites as a whole display a  $10^5$ -fold range in TL sensitivity, while the least metamorphosed, or type 3, ordinary chondrites display a  $10^3$ -fold range. Associated with this  $10^3$ -fold range in TL sensitivity are variations in the temperature at which the maximum TL emission occurs, and in the temperature range over which emission occurs<sup>3,4</sup>. We report here the results of annealing experiments on a little-metamorphosed (type 3.5) ordinary chondrite. The TL emission characteristics of the annealed samples show trends very similar to those observed in meteorites which have naturally been metamorphosed to various extents. The trends are also similar to those observed in annealing experiments on terrestrial albite<sup>5–7</sup> where the changes are associated with the low-to-high-temperature transformation. These data suggest that the TL phosphor in meteorites is feldspar and that TL can be used to estimate palaeotemperatures for little-metamorphosed and highly-unequilibrated meteorites.

About 1.5 g of the Allan Hills A77011 meteorite was ground ( $<100 \mu\text{m}$ ) and the magnetic fraction removed. Aliquots weighing 20 mg were then placed in high-purity quartz vials, evacuated to  $10^{-5}$  atmospheres, filled to atmospheric pressure with nitrogen and heat sealed. They were then placed in a wire-wound tube furnace at a variety of temperatures for 100 h. Two vials were annealed at each temperature. After annealing, the vials were allowed to cool in air, reaching room temperature after a few minutes. The TL induced by a  $250\text{-mCi } ^{90}\text{Sr } \beta$  source (absorbed dose 25 krad) was then measured 3–5 times. (Our apparatus and techniques are described in ref. 4). Irradiation times were chosen so as to give a signal-to-noise ratio of better than 10, usually it was  $>50$ . Data for each vial were averaged and the standard deviation calculated. The data for a given time

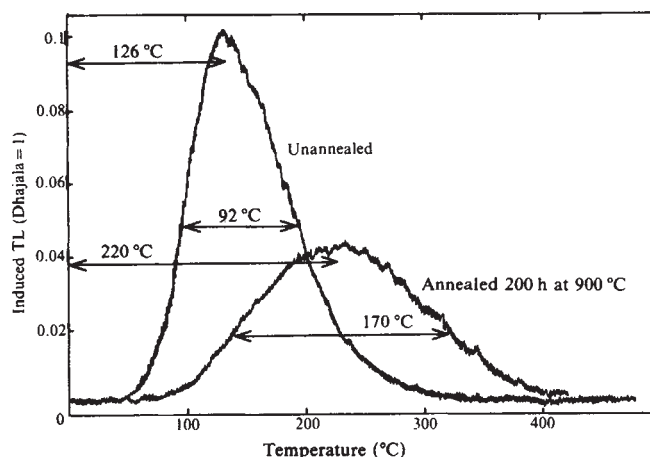
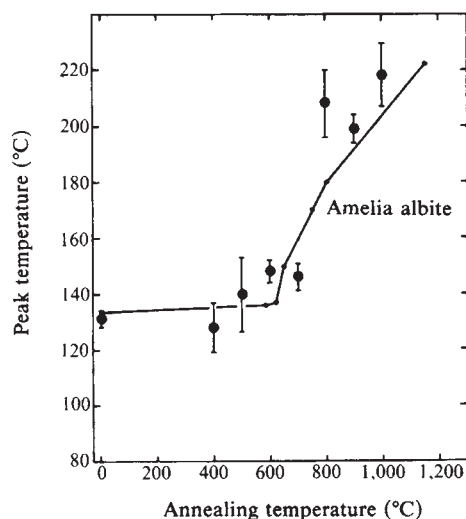


Fig. 1 Plots of the TL, induced by  $^{90}\text{Sr } \beta$  radiation, against temperature for an unannealed sample of the Allan Hills A77011 meteorite, and a sample which has been annealed at  $900^\circ\text{C}$  for 200 h. The annealing treatment has caused the TL peak, in fact, a composite of many smaller individual peaks, to broaden and move to higher temperatures.

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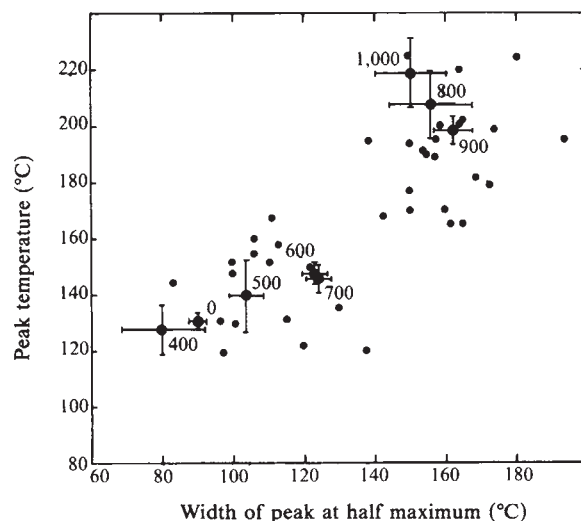


**Fig. 2** A plot of the temperature of the TL peak against the temperature at which each sample was annealed for 100 h. The error bars refer to  $\pm 1\sigma$  and each data point is the mean of two samples. Also shown are data from ref. 5 for a terrestrial albite from Amelia, Virginia, which was annealed for 24 h in a manner similar to that used here. The change in the TL emission characteristics of the Amelia albite were associated with the low-to-high temperature transformation, suggesting that the TL phosphor in meteorites is feldspar and that the shift in peak temperature on annealing may also be associated with changes in crystallography.

and temperature were averaged and the total uncertainty calculated by compounding the  $1\sigma$  uncertainties for the replicate measurements on each vial and for the standard, Dhajala to facilitate comparison between different laboratories.

Two curves for the TL induced by the  $\beta$  irradiation as a function of glow curve temperature are presented in Fig. 1. Ordinary chondrites display a broad band of TL at 100–300 °C which is composite of many smaller, individual peaks. The glow curves for an unannealed sample and for one of our most severely annealed samples are shown in Fig. 1. The TL peak is relatively sharp in the unannealed sample and appears at a lower temperature in the glow curve than the TL peak of the annealed materials. In the examples in Fig. 1, the annealed sample has its peak 94 °C higher in the glow curve, and its peak is 78 °C wider at half its maximum intensity, than that of the unannealed sample. Our annealed samples in general have glow curves which resemble one or other of the curves in Fig. 1, the less severely annealed samples resembling the unannealed curve—with some variability in the width—and the more severely annealed samples resembling the annealed curve.

Figure 2 shows a plot of the peak temperature of the TL emission as a function of the temperature to which the samples have been annealed. (Peak temperature is that at which the TL emission is at a maximum.) There is a slight indication of a  $<20$  °C increase in the position of the peak as the sample was annealed at temperatures up to 700 °C. The samples annealed at higher temperatures uniformly have TL peaks 80–100 °C higher than in the unannealed sample. We have also indicated on the plot Pasternak's data for samples of low albite from Amelia, Virginia<sup>5–7</sup>. Pasternak's samples were wrapped in Pt foil and annealed in He for 24 h. The agreement is striking and suggests that some change is occurring to the TL phosphor in both the feldspar and the meteorite between 600 and 800 °C. Pasternak performed X-ray diffraction studies on the Amelia albite and found that these changes in TL properties were associated with the transformation from the low-temperature (ordered) form to the high-temperature (disordered) form. However, the association was not a simple one as the TL changes occurred at faster rates than the order–disorder transformation; Pasternak suggests that the TL changes reflect crystallographic changes which precede the transformation, such as the formation



**Fig. 3** Peak temperature plotted against the width of the TL peak at half its maximum intensity for the present data (large symbols) and for type 3 ordinary chondrites (small symbols, data from refs 3 and 4). Alongside the present data points are numbers indicating the temperature (°C) at which the samples were annealed for 100 h; 0 indicates the unannealed samples. Errors are  $\pm 1\sigma$ ; two samples were run at each temperature. The meteorites show the same temperature–width relationship as the annealed samples, and the same tendency to bimodality, perhaps associated with the low- and high-temperature forms of the TL phosphor.

of defects. The low to high transformation temperature in albite or meteoritic feldspar (oligoclase in equilibrated meteorites, but unknown in others) is poorly known, but appears to be between 500 and 700 °C in albite<sup>8</sup>. Kinetic factors complicate the comparison of the meteorite and the Amelia annealing data with the feldspar phase diagram, but if the TL changes are associated with known crystallographic changes in the phosphor, the possibility exists that TL can be used for geothermometry.

Feldspar has not been petrographically observed in meteorites of petrologic type 3.5 or lower, except as occasional grains of anorthite usually referred to a 'primary'. However, transmission electron microscope observations of feldspar have been made in meteorites of types  $>3.7$  and feldspar is readily observable in types 5 and 6 where it seems to have formed by the devitrification of glass. Mineral separation experiments have shown that the TL phosphor is located in the low density, feldspar-rich component in equilibrated meteorites<sup>9</sup>. Therefore, we have previously suggested that the TL range in type 3 ordinary chondrites reflects progressive formation of feldspar by the devitrification of glass, and that the peak shape reflects the crystallography of the feldspar<sup>3</sup>. The present data are experimental evidence of that interpretation.

In Fig. 3 we compare the TL peak temperature with the width of the TL peak at half its maximum intensity. The data plot in two fields, the 800, 900 and 1,000 °C annealed samples have peaks at 200–220 °C and widths of 150–170 °C, while the unannealed and  $<700$  °C annealed samples have peaks at 130–150 °C and widths of 80–120 °C. There seems to be a correlation between peak temperature and width for unannealed and  $<700$  °C annealed samples. A  $\sim 50\%$  increase in width corresponds to a  $\sim 15\%$  increase in peak temperature. The change in peak width caused by the annealing treatment is, therefore, almost as marked as the change in peak position plotted in Fig. 2. We also plot in Fig. 3 data for 39 type 3 ordinary chondrites taken from refs 3 and 4. We have omitted meteorites with TL sensitivities  $<0.01$  because the TL-peak position and TL-peak width relationships break down for these very low TL meteorites where we suspect that a different TL phosphor becomes important. The meteorite samples display the same general trend as our annealed samples, and show a tendency for the peak width and peak position to produce two clumps which may be related



to the low- and high-temperature form of the TL phosphor. The data suggest that variations in thermal history are responsible for the peak position-peak width trends observed in type 3 ordinary chondrites<sup>3</sup> and that while some type 3 ordinary chondrites have experienced temperatures above 700 °C others have not. The trends in the chondrites are consistent with the idea that the least metamorphosed meteorites have a low-temperature form of feldspar as their TL phosphor. The implications of these data for our understanding of the metamorphic history of chondrites will be discussed elsewhere. However, one caveat is that our annealing times are very much less than the duration of metamorphism, but if, as we suspect, we are dealing with an equilibrium process, then laboratory measurements will be applicable. A study of the kinetics of the process is required.

Palaeothermometry of type 3 ordinary chondrites has always been a problem. The conventional approach of applying ther-

modynamic calculations to mineral pairs fails because the meteorites are non-equilibrium assemblages<sup>10</sup>. The oxygen-isotope thermometer works very well for petrologic types 4-6, but the heterogeneity problem is particularly acute for the type 3 chondrites because of the variable amounts of an <sup>16</sup>O-rich component<sup>11,12</sup>. We think that TL sensitivity may afford a new means of palaeothermometry in at least one rock type, the type 3 ordinary chondrites, where conventional methods fail. It might be usefully applied to other mildly metamorphosed rocks which contain feldspar as their dominant phosphor.

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## Seismic reflectors and unconformities at passive continental margins

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Vail and co-workers<sup>1-3</sup> have recently suggested, based on seismic reflection data available to Exxon, that more than 25 global unconformities occur in Mesozoic-Tertiary age passive continental margins around the world. These data have been used to subdivide the stratigraphical record into several cycles, each of which is controlled by oscillatory changes in sea-level. The resulting scheme has been used as a basis to predict the stratigraphy of passive margins where there is little or no well control<sup>4</sup> or where few reflector terminations can be found<sup>5</sup>. We report here four difficulties with this scheme: (1) during times of minimal continental ice cover changes in the relative rate of sea-level are generally not sufficient to cause unconformities, except in old, slowly subsiding, margins; (2) seismic and biostratigraphical resolution restricts the identification of unconformity-bounded stratigraphical sequences to relatively few in continental shelves; (3) sequence boundaries appear to be best defined on slopes or in regions of active tectonic tilting; and (4) limitations in the sequence analysis technique preclude its use in predictive stratigraphy, especially in shelf and slope regions of margins.

Watts and Steckler<sup>6</sup> used the 'backstripping' equation to determine eustatic changes in sea level from the subsidence history of commercial wells drilled in the margin off North America. Assuming point-wise isostasy this equation can be written:

$$Y = S^* \left( \frac{\rho_m - \rho_s}{\rho_m - \rho_w} \right) + W_d + \Delta_{SL} \left( \frac{\rho_m}{\rho_m - \rho_w} \right) \quad (1)$$

where  $Y$  is the tectonic subsidence of margin in water,  $W_d$  the water depth,  $\Delta_{SL}$  the sea level with respect to present,  $S^*$  the uncompacted sedimentary thickness, and  $\rho_m$ ,  $\rho_w$ ,  $\rho_s$  are mantle, water and sediment densities respectively. Equation (1) can be used to assess the ability of sea-level changes to cause a non-depositional or erosional unconformity. The condition for non-deposition or erosion in submarine or sub-aerial environments is given (assuming an absence of significant variations in the

rate of sediment supply to the shelf) by setting the rate of change in sediment thickness,  $S^*$ , to  $\leq 0$ . Equation (2) then becomes

$$\dot{\Delta}_{SL} \geq (\dot{Y} - \dot{W}_d) \left( \frac{\rho_m - \rho_w}{\rho_m} \right) \quad (2)$$

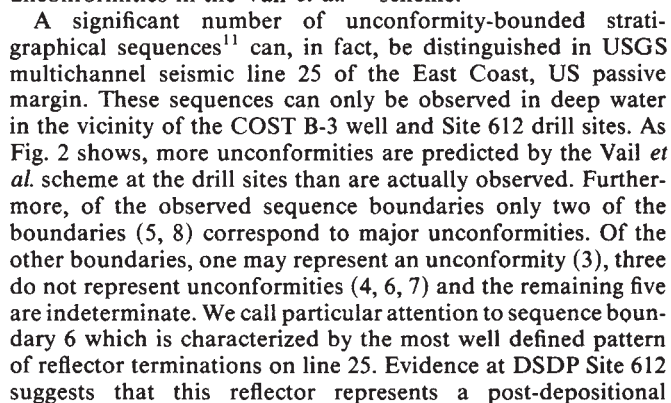
where the overdot refers to the derivative with respect to time. Both  $\dot{Y}$  and  $\dot{W}_d$  can be reasonably well approximated in the case of a passive margin. The variation of tectonic subsidence with time,  $\dot{Y}$ , follows straightforwardly, for the thermal subsidence stage of a margin<sup>7</sup>, from analytical expressions for the cooling by conduction of a plate or half-space<sup>8</sup>. In this study we assume for convenience the half-space approximation  $Y = Kt^{1/2}$ , where  $t$  is the age following rifting and  $K$  is a constant. Because of the assumption of a constant sediment supply rate, any deepening of the shelf ( $\dot{W}_d > 0$ ) will necessarily cause sediments to infill. Therefore, the condition for sea-level changes to cause an unconformity on a subsiding margin is given by:

$$\dot{\Delta}_{SL} \geq \frac{K(\rho_m - \rho_w)}{2\rho_m} t^{-1/2} \quad (3)$$

Minimum values of  $\dot{\Delta}_{SL}$  that are necessary to create an Oligocene unconformity in different age margins are summarized in Table 1. This shows that relatively small values of  $\dot{\Delta}_{SL}$  are required to form an Oligocene unconformity in an old passive margin while relatively large values are needed to form the same unconformity in a young margin.

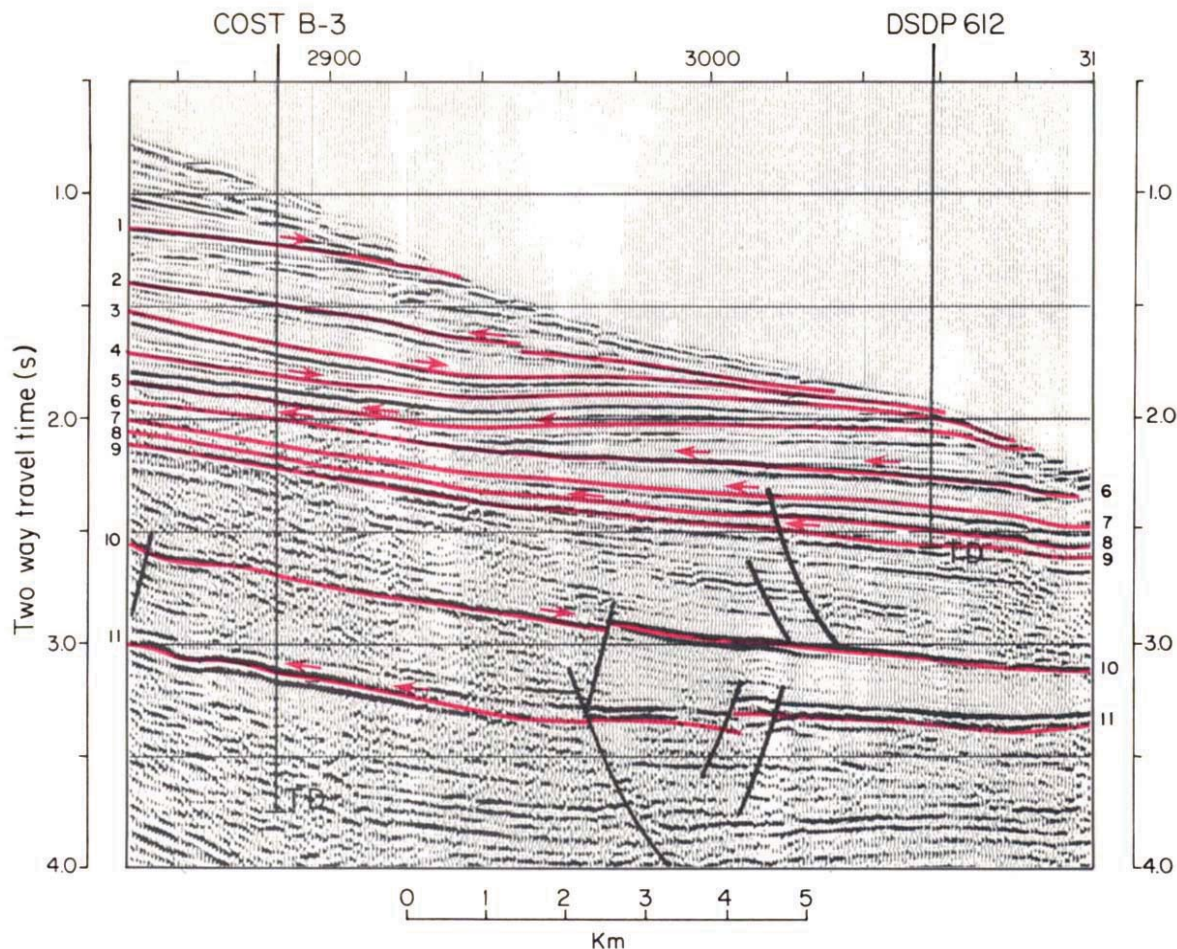
Pitman and Golovchenko<sup>9</sup> have pointed out that among the possible mechanisms, only continental glaciation could cause changes in  $\dot{\Delta}_{SL} \geq 1.2 \text{ cm kyr}^{-1}$ . Thus, in the absence of continental glaciation, an Oligocene unconformity would be expected at an old margin (for example, East Coast, US) but not at a young margin (for example, Norway). The occurrence of a prominent Oligocene unconformity in the margins of both the East Coast, US<sup>10</sup> and Norway<sup>11</sup> therefore implies continental glaciation during the Oligocene, a suggestion that is in accord with biogeographical evidence of the development of faunas and floras<sup>12</sup> as well as observations of enriched  $\delta^{18}\text{O}$  values in deep-sea sediments<sup>13</sup>. It is unlikely, however, that all the Cenozoic unconformities mapped by Vail *et al.*<sup>1-3</sup> are caused by glaciation. The problem then is to explain the many major unconformities that appear in the Vail *et al.* scheme.

If the sequence boundary technique of Vail *et al.*<sup>1-3</sup> is to be a useful stratigraphical tool, then the major unconformities should be easily recognized within the resolution of seismic and biostratigraphical data.



\* The total exponential subsidence was set equal to the  $t^{1/2}$  case.





**Fig. 2** USGS multichannel seismic line 25 (ref. 14) between shot-points 2847 to 3100. DSDP Site 612 (ref. 16) was drilled at 3558 on line 25. COST B-3 has been projected from 11 km to the north-east to 2885. The coloured reflectors 1-11 represent a preliminary interpretation consistent with the Vail *et al.*<sup>1-3</sup> scheme. Each sequence boundary is defined by reflector terminations (selected terminations shown by an arrow).

diagenetic front separating siliceous nannofossil chalk above from porcellanite below<sup>16</sup>. Although the origin of the front is unknown, it seems to be time-transgressive because it is observed just above the middle-early Eocene boundary at DSDP Site 612 and just below it at DSDP site 613 (ref. 16), drilled 11.5 km downslope of Site 612. The superposition of a diagenetic reflector at an angle to bedding plane reflectors may, therefore, have created the apparent pattern of onlap in Fig. 2. Reflectors should, in theory, be observed to continue across reflector 6. In practice, however, poor seismic resolution makes such an identification difficult.

It is, therefore, unclear which factors control the sequence boundaries on multichannel seismic line 25. We point out, however, that the sequence boundaries can only be easily recognized on the slope part of line 25 and that palaeoceanographic conditions may control at least part of these sequences.

Vail *et al.*<sup>1-3</sup> have repeatedly pointed out the limitations of the seismic sequence analysis technique. We have shown that these limitations may be made more severe by the pattern of subsidence that characterize passive margin development. Because the technique of predictive stratigraphy requires that all or most of the sequence boundaries can be easily recognized in an observed basin section, these limitations suggest caution if they are to be used in age correlation. For example, prominent seismic reflectors of the Barents shelf were 'dated' by Jørgensen and Navrestad<sup>4</sup> based on the Vail *et al.*<sup>1-3</sup> scheme. We caution against too literal an interpretation of these dates until the sedimentary sequences are actually drilled.

As a tool in predictive stratigraphy, it is commonly assumed that the Vail *et al.*<sup>1-3</sup> curve of changes in sea-level is applicable worldwide. We point out, however, that the nature of the control

on stratigraphical sequences is unclear. Thus, this assumption should be vigorously tested in the future.

Seismic sequence boundary techniques have proved a valuable tool in quantitative stratigraphy. We have pointed out that the number of sequence boundaries that can be attributed to changes in the rate of sea-level is probably less than previously thought. We have shown that slowly varying changes in sea level can cause major unconformities of wide extent on the shelf, particularly at relatively old passive margins. However, we do not expect that this mechanism can explain many unconformities on the slope. Alternative explanations, therefore, need to be sought for sequence boundaries on the slope, only some of which extend to the shelf. Future studies that include the effects of changes in sediment supply, ocean-wide tectonics, lithospheric mechanics, and palaeoceanographic effects offer the most promise of addressing the unconformity problem in the future.

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## Evolution of the lower continental crust: granulite facies xenoliths from the Eifel, West Germany

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The lower continental crust is a key region to understanding processes of crust–mantle differentiation and subsequent evolution of the crust. The region is metamorphosed into granulite or amphibolite facies<sup>1,2</sup>. Whereas felsic to mafic granulites with an average intermediate major element composition are typically observed in old exposed lower crustal terranes, mafic compositions are preponderant among granulite facies xenoliths transported by young alkaline basaltic volcanics and kimberlites<sup>1</sup>. Radiometric ages for granulite bulk rocks using the Rb–Sr or U–Pb methods are usually interpreted as metamorphic ages. In contrast, Sm–Nd ages obtained on such metamorphics are interpreted to reflect pre-metamorphic events<sup>3–7</sup>, although a case of severe disturbance of the Sm–Nd isotope system during granulite facies metamorphism has also been reported<sup>8</sup>. We present here Sm–Nd and Rb–Sr isotope data for a suite of mafic lower crustal xenoliths from the Hercynian belt. Our data suggest that the igneous precursors of these granulites differentiated from depleted mantle ~1.5 Gyr ago. In most of these granulites this evidence for a mid-Proterozoic age has been obliterated by a young (<172 Myr) metasomatic overprint by mantle derived fluids or melts.

The suite of lower crustal granulite facies xenoliths found in Pleistocene alkalic basaltic tuffs near Engeln/Eifel (Germany) has previously been investigated petrographically and chemically<sup>9,10</sup>. It has been suggested that these granulites are part of the Pleistocene deep crust and form a rather thin layer directly above the Moho<sup>9,10</sup> at ~30 km depth<sup>11</sup>. These xenoliths lack foliation typical of gneiss<sup>9</sup>. Their chemical composition is invariably 'basaltic' favouring an igneous rather than sedimentary origin of the granulite protoliths. On geological grounds the age of the granulite facies metamorphism must have been at least Caledonian<sup>9,10</sup>. A retrograde amphibolite facies metamorphic overprint evidenced by the formation of reaction coronas around garnets was attributed to crustal thinning and/or uplifting during the Hercynian orogeny<sup>9</sup>. The mineralogy of the granulites from Engeln as described by previous investigators is plagioclase ± clinopyroxene ± garnet ± orthopyroxene ± scapolite ± amphibole. Amphibole is ubiquitous in these nominally dry rocks and highly variable in modal abundance (<1–40%). Texturally the amphibole is clearly secondary in many cases where it formed at the expense of clinopyroxene whereas in other cases amphibole appears to be primary (for example, ref. 9 and own observations).

The results of our analysis of 10 granulites plus one upper mantle spinel peridotite from Engeln/Eifel are presented in Table 1. The presence of amphibole and a pronounced relative light rare earth element (REE) enrichment, classify this mantle xenolith as 'metasomatized', similar to a suite of spinel peridotites from Dreiser Weiher/Eifel<sup>12</sup>.

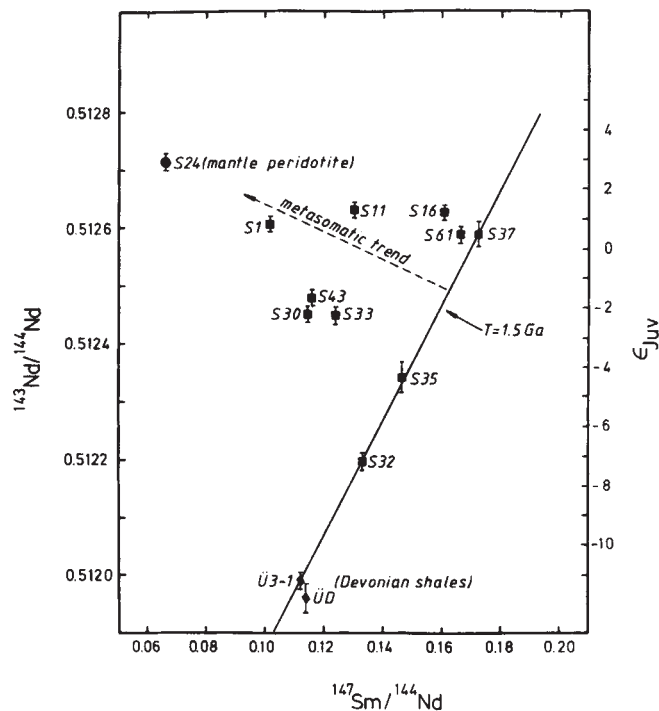


Fig. 1 Sm–Nd isotope evolution diagram for granulites, one peridotite and sediments. An age of ~1.5 Gyr is inferred for a crust forming event involving depleted mantle with  $\epsilon_{Juv}(T) \sim +5$ . The arrow indicates the direction of Nd isotopic displacement of parts of the lower crust underneath the Eifel towards mantle values due to metasomatism with mantle derived fluids. This also led to a further light REE enrichment.

dotites from Dreiser Weiher/Eifel<sup>12</sup>. To characterize the local upper crust, we have also analysed one small (~2 cm) sedimentary inclusion (Lower Devonian?) from a basanite flow near Uedersdorf/Eifel as well as one sample of Lower Devonian shale outcropping nearby. The purpose was to possibly obtain information on the crustal residence time of the igneous precursors of these sediments by using the Sm–Nd isotopic system. This follows the approach of McCulloch and Wasserburg<sup>27</sup> and O'Nions and coworkers<sup>21,28</sup>.

The Sr isotopic composition of the mantle peridotite S24 is similar to that reported previously for amphibole-bearing spinel peridotites from Eifel<sup>13</sup>.  $^{87}\text{Sr}/^{86}\text{Sr}$  of the granulite xenoliths ranges from typical mantle (~0.7036) to crustal values (~0.7095). Rb concentrations and Rb/Sr ratios are among the lowest reported for granulite facies metamorphics so far. The highest Rb concentrations are found in the granulites with the highest modal abundances of amphibole. Due to the overall low Rb/Sr ratios  $^{87}\text{Sr}/^{86}\text{Sr}$  is essentially frozen, and thus does not allow time constraints to be placed on metamorphic or other events. While the small Rb/Sr ratios of the granulites with low  $^{87}\text{Sr}/^{86}\text{Sr}$  may have been established during formation of their igneous precursors, high  $^{87}\text{Sr}/^{86}\text{Sr}$  require that samples like S32 evolved with high Rb/Sr for a considerable time. These data therefore support interpretations similar to those applied to Archaean high-grade metamorphic terranes that Rb, among other elements, can be highly mobile and partly lost during granulite facies metamorphism<sup>14–16</sup>. The two upper crustal sediments have high  $^{87}\text{Sr}/^{86}\text{Sr}$  and Rb/Sr ratios, attesting to the generally enriched nature of the heavy alkalies in the upper relative to the lower crust.

The Sm–Nd isotopic results given in Table 1 are plotted in Fig. 1 as an isotope evolution diagram. For the granulites two trends can be separated. One trend runs from S35 or from S37 towards the metasomatized spinel peridotite S24, with an inverse correlation between Sm/Nd and  $^{143}\text{Nd}/^{144}\text{Nd}$ . More specifically, the direction of the trend is from typical crustal Nd isotopic



**Table 1** Analysis of Engeln/Eifel samples

|            |      | Rb        | Sr     | <sup>87</sup> Rb/ <sup>86</sup> Sr | <sup>87</sup> Sr/ <sup>86</sup> Sr† | Sm     | Nd        | <sup>147</sup> Sm/ <sup>144</sup> Nd | <sup>143</sup> Nd/ <sup>144</sup> Nd† | $\epsilon_{\text{JUV}}(0)‡$ |
|------------|------|-----------|--------|------------------------------------|-------------------------------------|--------|-----------|--------------------------------------|---------------------------------------|-----------------------------|
|            |      | [p.p.m.]* |        |                                    |                                     |        | [p.p.m.]* |                                      |                                       |                             |
| Granulites | S1   | 1.05      | 690.7  | 0.0044                             | 0.704922 ± 26                       | 7.16   | 42.6      | 0.1015                               | 0.512608 ± 14                         | +0.8 ± 0.3                  |
|            | S11  | 0.73      | 577.0  | 0.0037                             | 0.703639 ± 26                       | 1.290  | 6.01      | 0.1298                               | 0.512632 ± 14                         | +1.3 ± 0.3                  |
|            | S16  | 0.33      | 407.6  | 0.0023                             | 0.705245 ± 26                       | 1.194  | 4.50      | 0.1604                               | 0.512628 ± 14                         | +1.2 ± 0.3                  |
|            | S30  | 3.4       | 916.0  | 0.0107                             | 0.705790 ± 28                       | 5.82   | 30.7      | 0.1145                               | 0.512453 ± 14                         | -2.2 ± 0.3                  |
|            | S32  | 0.94      | 1325.0 | 0.00204                            | 0.709478 ± 28                       | 8.07   | 36.9      | 0.1323                               | 0.512198 ± 14                         | -7.2 ± 0.3                  |
|            | S33  | 4.64      | 606.0  | 0.0222                             | 0.704568 ± 26                       | 9.72   | 47.5      | 0.1238                               | 0.512451 ± 14                         | -2.2 ± 0.3                  |
|            | S35  | 1.25      | 266.1  | 0.0136                             | 0.705460 ± 26                       | 6.02   | 24.98     | 0.1458                               | 0.512343 ± 26                         | -4.4 ± 0.5                  |
|            | S37  | 0.210     | 406.2  | 0.00149                            | 0.705207 ± 26                       | 1.500  | 5.27      | 0.1720                               | 0.512590 ± 22                         | +0.5 ± 0.4                  |
|            | S43  | 7.1       | 759.0  | 0.0270                             | 0.704501 ± 32                       | 9.27   | 48.4      | 0.1159                               | 0.512480 ± 14                         | -1.7 ± 0.3                  |
|            | S61  | 0.40      | 157.8  | 0.0073                             | 0.704626 ± 28                       | 3.47   | 12.66     | 0.1659                               | 0.512591 ± 14                         | +0.5 ± 0.3                  |
| Peridotite | S24  | 0.051     | 61.0   | 0.0024                             | 0.704029 ± 26                       | 0.2924 | 2.678     | 0.0660                               | 0.512714 ± 16                         | +2.9 ± 0.3                  |
| Sediments  | ÜD   | 152       | 119.6  | 3.69                               | 0.735535 ± 26                       | 7.45   | 39.7      | 0.1134                               | 0.511961 ± 26                         | -11.8 ± 0.5                 |
|            | Ü3-1 | 197       | 146.4  | 3.90                               | 0.731533 ± 42                       | 7.90   | 42.7      | 0.1118                               | 0.511989 ± 16                         | -11.3 ± 0.3                 |

Isotopic fractionation was corrected to <sup>86</sup>Sr/<sup>88</sup>Sr = 0.1194 and <sup>148</sup>Nd/<sup>144</sup>Nd = 0.241572; Nd was measured as NdO<sup>+</sup>; <sup>87</sup>Sr/<sup>86</sup>Sr = 0.71026 measured for NBS987 Sr and <sup>143</sup>Nd/<sup>144</sup>Nd = 0.511859 for the La Jolla Nd standard.

\* Uncertainties for concentrations: [Rb] ± 0.5%, [Sr] < ± 0.2%, [Sm] and [Nd] < ± 0.1%.

† In most instances actual in-run errors are smaller than quoted; minimum errors given are derived from external precision of standard measurements which are 37 and 27 p.p.m. for <sup>87</sup>Sr/<sup>86</sup>Sr and <sup>143</sup>Nd/<sup>144</sup>Nd, respectively.

‡ Present-day <sup>143</sup>Nd/<sup>144</sup>Nd for JUVINAS is 0.512566 ( $\epsilon_{\text{JUV}}(0) = 0$ ).

$$\epsilon_{\text{JUV}}(0) = \left[ \frac{{}^{143}\text{Nd}/{}^{144}\text{Nd}_{\text{sample}}^{\text{measured}}}{{}^{143}\text{Nd}/{}^{144}\text{Nd}_{\text{JUVINAS}}^{\text{present}}} - 1 \right] \times 10^4$$

values to values found for metasomatized mantle spinel peridotites (see also ref. 13). Along this trend the modal content of amphibole increases from <5% in S35 to 30–40% in S30. Large ion lithophile element-rich fluids or liquids originating in the mantle probably reacted with the granulites to a variable degree. This trend, therefore, is the result of extensive metasomatism which also is responsible for further light REE enrichment. The time at which the metasomatic modification of an originally dry into hydrous (amphibole and/or phlogopite containing) upper mantle occurred was previously estimated for this region to be <200 Myr ago<sup>13</sup>.

Sm–Nd data for plagioclase, clinopyroxene and garnet from granulite S30 (see Table 2 and Fig. 2) form an isochron with a slope corresponding to an age of 172 ± 5 Myr. The data for amphibole as well as the bulk rock, however, are clearly above the isochron. They are displaced towards values for amphibole-bearing mantle peridotites from the Eifel but not towards values for the crust in this area which, at a similar Sm/Nd ratio, would be below the isochron. The same argument holds true for <sup>87</sup>Sr/<sup>86</sup>Sr. Amphibole (~0.7054) is displaced towards mantle values relative to clinopyroxene and plagioclase (~0.7061 for both minerals). The data point for S30-bulk in Fig. 2 is almost identical to that for S30-amphibole because by mass balance the amphibole accounts for some 2/3 of this rock's total Nd content. This strongly supports our interpretation that the formation of amphibole in these granulites is due to metasomatism by mantle-derived fluids. The age of this mineral isochron can be regarded as a maximum age for the formation of amphibole and, hence, metasomatism in the lower crust.

The evidence for metasomatic modification of the lower crust clearly indicates that any information on the timing of pre-metasomatic events can only be obtained from those granulite xenoliths which are farthest removed from values for metasomatized mantle in Fig. 1, that is, granulites S32, S35 and S37. These

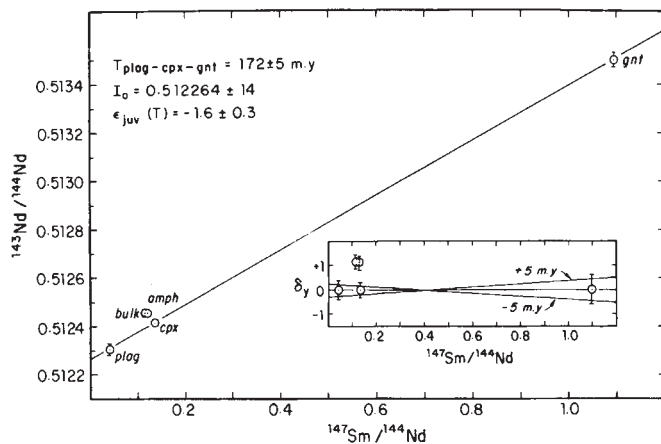
three data points plot along a line with a slope corresponding to an age of 1.5 ± 0.1 Gyr and an initial  $\epsilon_{\text{JUV}}(T) = +4.7 ± 1.9$ . We interpret this as evidence that part of the lower crust beneath the Rhenish Shield differentiated from depleted mantle at around this time. Of course, neither the igneous age nor the initial  $\epsilon_{\text{JUV}}$  of these rocks are tightly constrained by the three data points. Nevertheless, the isochron appears to be real since no mixing relationships can be derived from our isotopic and elemental abundance data. This evidence strongly contradicts the still widely held view that the continental crust in the Hercynian belt in Central Europe is not older than ~700 Myr (refs 17, 18). Additional support comes from U–Pb studies on zircons from the Hercynian fold-belt in Europe which yield even older ages, between 1.9 and 2.2 Gyr (refs 18, 19).

From Fig. 1 it is apparent that the data for the Devonian sediments ÜD and Ü3-1 plot on or close to the line through the data points for granulites S37, S35 and S32, that is their Sm–Nd isotope systematics are consistent with their having about the same 'age' as the granulites. It is worth noting here that loess deposits from Central Europe have Sm–Nd depleted mantle model ages around 1.5 Gyr (ref. 20). Fine-grained clastic sediments from the British Isles south of the late Precambrian to early Palaeozoic Iapetus Ocean have very uniform Sm–Nd depleted mantle model ages around 1.7 Gyr (ref. 21), that is, only slightly higher than the age obtained by us for the Eifel rocks. Both types of sediments probably provide good averages of their upper crustal sources with respect to Sm/Nd ratios, and <sup>143</sup>Nd/<sup>144</sup>Nd compositions which can be used to estimate their crustal residence ages. Although it may be fortuitous that the sediment data fall on or close to the line through the data for the granulites in Fig. 1, we consider that the igneous precursors of granulites and sediments have been extracted from the mantle at around the same time, that is towards the end of the ~1.6–2.0 Gyr tectonothermal event which affected wide parts of the Baltic

**Table 2** Sm–Nd results for separated minerals from granulite S30

|               | Sm       | Nd    | <sup>147</sup> Sm/ <sup>144</sup> Nd | <sup>143</sup> Nd/ <sup>144</sup> Nd | $\epsilon_{\text{JUV}}(0)$ |
|---------------|----------|-------|--------------------------------------|--------------------------------------|----------------------------|
|               | [p.p.m.] |       |                                      |                                      |                            |
| Amphibole     | 11.81    | 59.0  | 0.1211 ± 1                           | 0.512456 ± 14                        | -2.1 ± 0.3                 |
| Clinopyroxene | 4.97     | 22.65 | 0.1326 ± 1                           | 0.512415 ± 14                        | -2.9 ± 0.3                 |
| Garnet        | 1.824    | 1.007 | 1.095 ± 1                            | 0.513499 ± 30                        | +18.2 ± 0.6                |
| Plagioclase   | 0.1831   | 2.804 | 0.03946 ± 8                          | 0.512306 ± 24                        | -5.1 ± 0.5                 |

Conditions and methods as in Table 1.



**Fig. 2** Sm-Nd isochron diagram for separated minerals from granulite S30 (plog, plagioclase; cpx, clinopyroxene; amph, amphibole; gnt, garnet). All minerals have been hand-picked and etched in dilute HCl and HF prior to dissolution to ensure absence of any contaminants. Inset shows deviation ( $\delta y$ ) in parts of 10,000 of the data from the best fit-line through plagioclase, clinopyroxene and garnet.

and Laurentian Shields<sup>22</sup>. Our argument, particularly with regard to involvement of depleted mantle, is further strengthened by the high value of  $\epsilon_{\text{JUV}}(T)$ . This value is practically identical to the  $\epsilon_{\text{JUV}}(T)$  indicated for depleted mantle underlying parts of the North American continent<sup>23,24</sup> and Greenland<sup>24</sup> and suggests a relatively large-scale Nd isotopic homogeneity in the upper mantle at around this time. Our isotope data for the Eifel samples add further constraints to the understanding of the derivation through time of continental crust from progressively depleted mantle.

Metamorphic events in the lower crust under the Eifel do not seem to have severely affected the Sm-Nd isotope systematics, in agreement with previous suggestions that the Sm-Nd clock can survive even granulite facies metamorphism<sup>4-7</sup>. The age of the event which metamorphosed the deep crust under the Eifel into granulite facies cannot be disclosed by our Sm-Nd data. However, a Caledonian or older age of the high-grade metamorphism<sup>9,10</sup> cannot be confirmed by our Sm-Nd or Rb-Sr data. In contrast, the results from the mineral isochron (Fig. 2) require Mesozoic ages to be considered as well.

We have suggested that relatively young metasomatism has transported large ion lithophile elements (probably along with volatiles like  $\text{CO}_2$  or  $\text{H}_2\text{O}$ ) from the mantle into  $\sim 1.5$  Gyr-old lower crust leading to the formation of amphibole in previously dry granulites. In the case of the Eifel, this has resulted in shifts from crustal to near bulk Earth Sr and Nd isotopic compositions for the majority of the analysed granulites (average  $^{87}\text{Sr}/^{86}\text{Sr} \sim 0.7048$ , average  $\epsilon_{\text{JUV}} \sim -1$  for all granulites not lying on the isochron in Fig. 1). This shift represents an isotopic rejuvenation of the lower crust.

Metasomatism is well known from mantle xenoliths of world-wide occurrence, making depleted mantle fertile with respect to its capability to produce alkalic basaltic melts<sup>25</sup>. If, as described here for the Eifel, metasomatic modification by mantle derived fluids or melts is widespread in the lower crust, at least in regions of continental rifting or uplift, it will become necessary to view certain aspects of the evolution of the continental crust (for example, the origin of felsic plutonic rocks, with mantle initial  $^{87}\text{Sr}/^{86}\text{Sr}$  and  $^{143}\text{Nd}/^{144}\text{Nd}$  values) also within the context of derivation from metasomatized lower crust<sup>26</sup>.

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## Relationship between continental area and elevation

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Continental hypsographic curves, derived from data gathered by Kossinna<sup>1</sup>, show a similarity of shape that suggests a correlation between continental area (including the continental shelf) and average height<sup>2,3</sup>. I have considered the varied geological histories of the continental blocks and suggest here that this relationship is not fortuitous, and that changes in continental groupings will have profound effects not only on ocean volumes, and hence sea level, but also on the three-dimensional shapes of the continental blocks.

Harrison *et al.*<sup>2</sup> consider that the apparent link between area and elevation can best be expressed in the form

$$h = -0.4623 + 0.3731 \log_e A \quad (1)$$

where  $h$  is the height (in km) above the continental shelf edge (taken as 200 m below sea level), and  $A$  (in  $\text{Mm}^2$ ) is the continental area. This expression was used to derive a theoretical hypsographic curve for Pangaea to explain the lower than otherwise expected areal flooding during eustatic rises of sea level.

This expression was calculated using data from Kossinna<sup>1</sup>, but Harrison *et al.*<sup>3</sup> have recently produced more detailed data that would modify this relationship. From a geological point of view the geographer's continental masses have to be regrouped to conform with what is known of crustal movements (see refs 4, 5). The major changes necessary involve the combination of the values for Europe and most of Asia, which appear to have acted as a block for the Mesozoic and Cenozoic, and the removal from Asia of the Arabian Peninsula, which seems to belong more to the African continent. The collision zone of India and Eurasia is complex, and may include other continental fragments<sup>6</sup> and much underthrusting. The data of Harrison *et al.*<sup>3</sup> suggest how the volume of underthrust crust may be estimated, but it is not possible to resolve this into a simple set of area/height data for a separate India. I have, therefore, included India with Eurasia. Details of the hypsographic curves for the rock surfaces of Antarctica and Greenland are not known to



**Table 1** Elevation and area data for present continental masses

| Continent     | Average height (m) | Area ( $10^{12} \text{ m}^2$ ) | Average thickness (km) | Volume ( $10^{12} \text{ m}^3$ ) |
|---------------|--------------------|--------------------------------|------------------------|----------------------------------|
| Eurasia       | 834                | 59.92                          | 37.2                   | 2,227,750                        |
| Afrabia       | 824                | 34.82                          | 37.1                   | 1,292,500                        |
| North America | 699                | 26.74                          | 36.3                   | 971,250                          |
| South America | 723                | 20.10                          | 36.5                   | 733,000                          |
| Australia     | 443                | 11.12                          | 34.7                   | 385,750                          |
| Total         |                    | 152.70                         |                        | 5,610,250                        |
| Antarctica    |                    | 13.68                          |                        | 484,250                          |
| Greenland     |                    | 2.56                           |                        | 84,500                           |
| Total         |                    | 168.94                         |                        | 6,179,000                        |

Data are based in part on ref. 3. Average heights are above 200 m below present sea level. Average thickness calculated as shown in the text. Volumes given to the nearest  $250 \times 10^{12} \text{ m}^3$ . Data for Antarctica and Greenland are less reliable than that for the other continental masses.

any great accuracy (estimates of the average elevation of the Antarctic ice cap range from 785 to 2,200 m), and therefore are not used in the calculations below. Harrison *et al.*<sup>3</sup> have separated Madagascar from the rest of Africa. There is still controversy over the nature of the Mozambique channel, and it also looks as if part of the Madagascar Ridge may be thin continental crust<sup>7</sup>, which would change the average elevation of the Madagascar block considerably. These data have, therefore, also been left out of the calculations.

The average total thickness of a continental block was calculated assuming that the blocks are in approximate isostatic equilibrium (Fig. 1). Taking average values for the density of continental crust ( $2.782 \text{ g cm}^{-3}$ ), oceanic crust ( $2.9 \text{ g cm}^{-3}$ ) and seawater ( $1.03 \text{ g cm}^{-3}$ )<sup>8</sup> it is possible to derive a simple formula relating the average height of a block to the average depth of its root. For isostatic equilibrium

$$d_w h_4 + d_c h_2 + d_m h_3 = d_c h_1 + d_c h_2 + d_c h_3$$

or

$$h_3 = 5.37 h_1 - 0.398 \quad (2)$$

It is also possible to derive an expression for the thickness of continental crust below a continent of average height 200 m below sea level. Additional assumptions are the average depth of old oceanic crust (6,400 m below sea level)<sup>9</sup> and the average thickness of oceanic crust (6,600 m)<sup>10</sup>. For isostatic equilibrium

$$d_c (h_5 - h_4) + d_c h_6 + d_c h_7 = d_w (h_5 - h_4) + d_o h_6 + d_m h_7$$

or

$$h_7 = 19.466 \text{ km}$$

then

$$h_2 = 32.266 \text{ km}$$

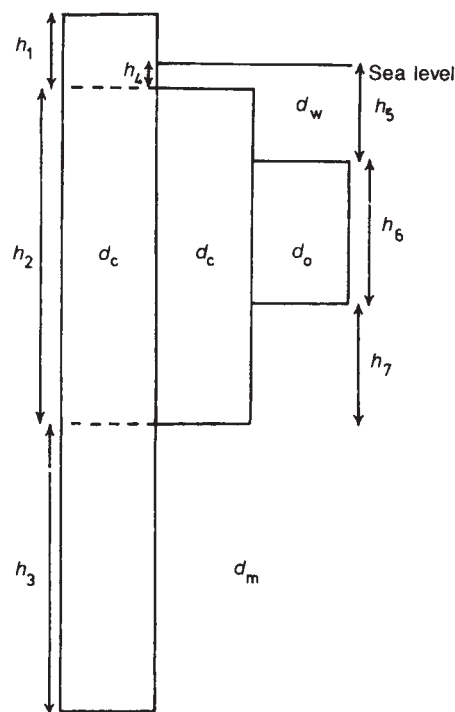
Thus, the total thickness of continental crust of a continent of average height  $h$ , will be given by the expression

$$\text{Total thickness} = 31.868 + 6.37 h \text{ km} \quad (3)$$

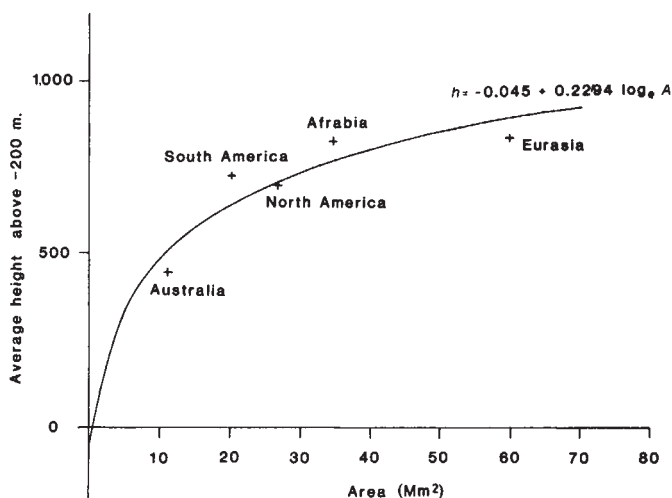
Table 1 lists the revised average height and area figures, the total thickness calculated using equation (3), and the total volume of continental crust for each block. Figure 2 shows average height plotted against area for the blocks with good data, with a curve fitted to these points. Figure 2 suggests that the relationship is better expressed as

$$h = -0.045 + 0.2294 \log_e A \quad (4)$$

Both the curve fitted to the data in Fig. 2, and the expression derived by Harrison *et al.*<sup>2</sup>, have an interesting consequence. If the relationship has held throughout geological time, then the average elevation of the supercontinents must have been greater than that of the fragments we now see. And if the average height



**Fig. 1** Blocks in isostatic equilibrium.  $d_i$ , Density of material  $i$ , where c, continental crust; m, mantle; o, oceanic crust; w, seawater.



**Fig. 2** Relationship between average height and area for those continental blocks with reliable data. The regression line is fitted by least squares.

was greater, the depth of the continental crust must also have been greater to preserve isostatic equilibrium. Unless we invoke changes in the volume of continental crust in parallel with the formation and splitting of continental blocks, this implies changes with time in the area of the globe covered by continental crust. There are sound geochemical arguments showing that the total volume of continental crust has remained almost constant for at least the Phanerozoic, and probably much longer, so it is possible to plot the relationship of volume to area and to calculate the changes in total area of the globe covered by continental crust with different continental groupings.

The relationship of volume to area is given by

$$\begin{aligned} V &= (31.868 + 6.37 h) A \\ &= (31.581 + 1.4613 \log_e A) A \end{aligned} \quad (5)$$

This relationship is plotted on Fig. 3. The numerical values in the expression are dependent on the values for densities and

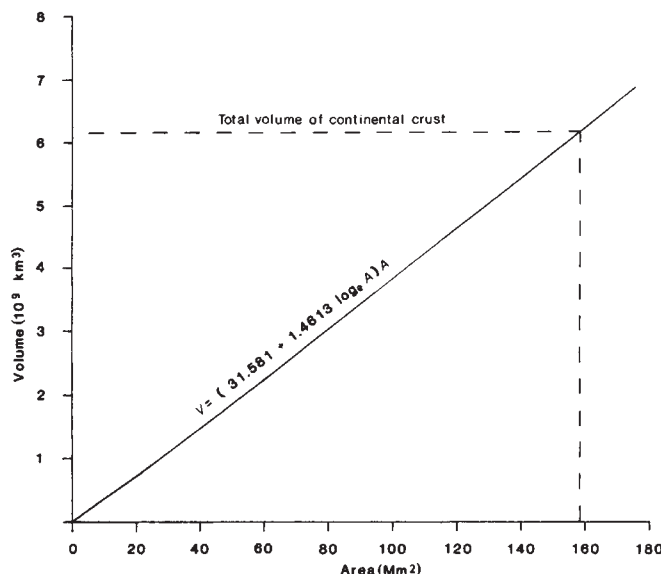


Fig. 3 The theoretical relationship between the volume of a continental block and its surface area.

depths, but slight variation in these values does not affect the conclusions drawn below. From equations (4) and (5) it is possible to calculate theoretical values for the average height and volume of Antarctica and Greenland. These are listed in Table 1, and used simply because they will act in the same way as the other continental blocks.

Figure 3 shows that if all the continental crust formed one block, the total area covered would be  $\sim 158.5 \text{ Mm}^2$ . This may represent the situation in the early Mesozoic when Pangaea was extant. This is considerably smaller than the present total area covered by continental crust which is about  $168.9 \text{ Mm}^2$  (Table 1). This implies that the volume of the ocean basins in the early Mesozoic was  $\sim 66.6 \times 10^6 \text{ km}^3$  more than it is at present, before allowing for isostatic adjustment due to smaller water load. This is equivalent, after isostatic adjustment, to a sea level some 125 m below the present level.

As Pangaea broke up into smaller fragments these would have altered their configuration to conform with equation (4) that is, the area of a given fragment would increase as its height (and thickness) decreased until it reached a new stable state. Such changes in the area of the continental fragments would have affected the volume of the ocean basins, not only directly, but also by increase in subduction rates and/or decrease in the rate of creation of new crust at ridges, with associated changes in sea level. It may be possible to estimate the rates involved in this flattening process by looking at the history of small fragments of continental crust that have become detached from larger units, as the model suggests that their average height should be much less than that of the parent unit. Berggren<sup>11</sup>, for example, suggests, on the basis of microfauna from JOIDES samples, that the Rockall Bank area sank by  $\sim 600 \text{ m}$  in a time span of some 6 Myr in the Palaeogene. The geological history of some of the microcontinents in the South European Tethys may record the opposite process, as may the late Cenozoic history of the Indian continental crust.

In addition to predicting changes in sea level the model shows that a large continent will have a smaller area of shallow sea than several smaller continents with a total area equal to the large continent. A rough calculation based on a continent of area  $158.5 \text{ Mm}^2$  suggests a shallow sea area (that is down to about 200 m below the sea level) of about  $13 \text{ Mm}^2$ , which is about half the area of modern shallow seas. This has important consequences for evolutionary studies.

The model poses problems for palaeocontinental reconstruction, as it predicts different depth compensation histories for

different sized fragments, as well as expansion of the fragments. This may go some way to resolving problems associated with expanding Earth hypotheses, as it suggests that the continents have generally increased their areal extent since the breakup of Pangaea. The Palaeozoic history of the continental blocks should, in general, show the opposite effect.

Cogley<sup>12</sup> has produced an independent reassessment of continental area and height relationships. He recognizes more continental blocks than those used above, suggesting that the total area of continental crust is considerably greater, but his data suggest a similar relationship between mean height and continental area. Modal heights do not show such a simple relationship, suggesting that calculations of shallow sea areas are not as straightforward as might be expected.

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## A lower crustal origin for massif-type anorthosites

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The origin of batholith-sized masses of plagioclase-rich crustal rocks, mainly Proterozoic in age, has mostly been ascribed to aluminous magmas of mantle derivation. Here we propose that such masses are derived by partial melting of an aluminous lower continental crust, which has developed following extraction of a granitic upper crust by intra-crustal melting. This segregation of upper and lower crusts occurred principally in the late Archaean. As the lower crust was depleted in heat-producing elements during the initial melting to produce granites, an external source of heat is required to produce Proterozoic massif-type anorthosites. We speculate that this heat resulted from the continental crust moving over a mantle hotspot or line plume.

Anorthosites may be classified broadly into two types: Archaean and massif. The Archaean anorthosites typically occur as components of layered igneous intrusions<sup>1</sup> of moderate size and are clearly of mantle origin. Plagioclase compositions lie in the range  $An_{80-95}$ . In contrast, the massif-type anorthosites which occur as large batholith masses up to  $30,000 \text{ km}^2$  in outcrop area, present major petrological and geochemical problems<sup>2-9</sup>. Plagioclase compositions are intermediate, typically  $An_{40-60}$  and  $Mg/(Mg+Fe)$  ratios are in the range 40–50. Their isotopic characteristics are enigmatic<sup>9</sup>. They are notably depleted in Rb with very low Rb/Sr ratios. Typical initial  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios are low but variable, ranging from 0.7025 to 0.7070, unsupported in many examples by the present Rb/Sr ratios.  $\epsilon_{\text{Nd}}$  values are even more erratic varying from enriched (–6) to depleted (+4) sources<sup>9</sup>. The bulk composition displays enrichment in Eu relative to the other chondrite-normalized rare-earth elements (REE) (Fig. 1). In addition to their petrological and geochemical



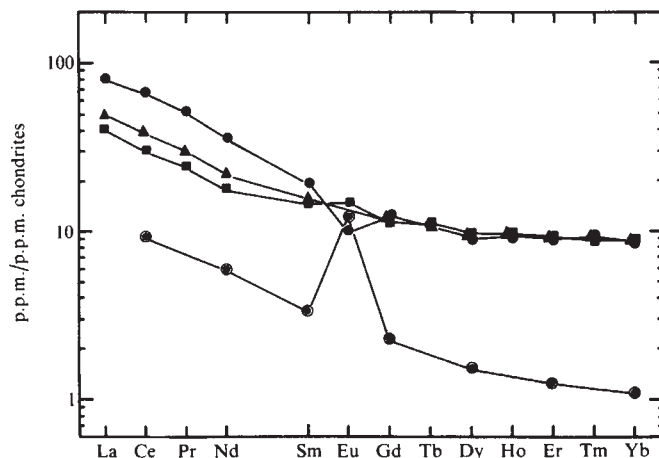


Fig. 1 Chondrite-normalized REE diagram for upper (●), lower (■) and total (▲) continental crust<sup>12</sup>. Also shown is a typical Proterozoic anorthosite pattern (○).

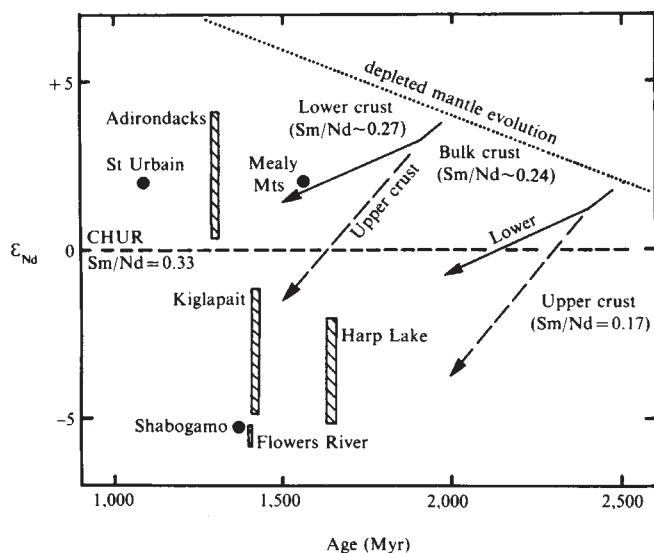


Fig. 2 Fractional deviations from CHUR (chondritic reservoir) in parts per  $10^4$  of initial  $^{143}\text{Nd}/^{144}\text{Nd}$  ( $\epsilon_{\text{Nd}}$ ) in Proterozoic anorthosites<sup>1,8</sup>. Also shown are the evolutions of depleted mantle and lower, upper and bulk crust. The range in  $\epsilon_{\text{Nd}}$  values of the anorthosites is compatible with a 300–500 Myr residence period in the lower crust.

peculiarities, they are primarily a Proterozoic phenomenon, mostly intruded between about 1,700 and 900 Myr ago. Massif-type anorthosites also seem to be related to unique tectonic conditions during the Proterozoic, occurring at sites of incipient or failed continental rifting<sup>5,8,10,11</sup>.

We believe that the high Al content, the low An content of the plagioclase and the low Mg/(Mg + Fe) of these rocks is incompatible with a mantle source for Proterozoic massif anorthosites, and now suggest that they are derived from the lower crust. In the model<sup>12–14</sup> that we adopt for the development of the continental crust, a massive increase in continental volume occurred between about 3,000 and 2,500 Myr followed closely by a large intra-crustal melting event which produced enough K-granites to dominate the composition of the upper crust. Thus, the lower crust becomes both dry and depleted in K, Rb, U, Th and the other elements concentrated in granitic melts (Table 1); it also becomes enriched in Al, Sr and Eu (Fig. 1), so that there is an overall enrichment in Eu, relative to chondrite-normalized REE patterns, and the development of low Rb/Sr ratios.

The model composition for the bulk lower crust is given in Table 1. This shows a low quartz and orthoclase content, due

Table 1 A model composition of the lower crust<sup>12</sup>

|                                | (%)   |    | (p.p.m.) |
|--------------------------------|-------|----|----------|
| SiO <sub>2</sub>               | 54.4  | Cs | 0.1      |
| TiO <sub>2</sub>               | 1.0   | Ba | 150      |
| Al <sub>2</sub> O <sub>3</sub> | 16.1  | Rb | 5.3      |
| FeO <sub>T</sub>               | 10.6  | Sr | 230      |
| MgO                            | 6.3   |    |          |
| CaO                            | 8.5   | La | 11       |
| Na <sub>2</sub> O              | 2.8   | Ce | 23       |
| K <sub>2</sub> O               | 0.33  | Nd | 12.7     |
| Σ                              | 100.0 | Sm | 3.17     |
|                                |       | Eu | 1.17     |
| Norm                           |       | Gd | 3.3      |
| Q                              | 3.7   | Tb | 0.59     |
| Or                             | 2.0   | Dy | 3.6      |
| Ab                             | 23.7  | Er | 2.2      |
| An                             | 30.4  | Yb | 2.2      |
| Di                             | 9.8   | Lu | 0.32     |
| Hy                             | 28.6  | Th | 1.06     |
| Il                             | 1.9   | U  | 0.28     |
|                                |       | Hf | 2.1      |
|                                |       | Ta | 0.6      |
|                                |       | Cr | 235      |
| Mg/Mg + Fe                     | 0.51  | Sc | 36       |
|                                |       | Ni | 135      |
|                                |       | Co | 35       |

to previous extraction of granitic melt. COCORP studies<sup>15</sup> show that the composition of the lower crust is complex. Accordingly, many regions of the lower crust will be even more basic (as shown by many lower crustal xenoliths) and be an appropriate source for the mafic minerals in the massif anorthosites. From our model we predict that anorthosites should possess mineralogical, isotopic and chemical variations which reflect the lower crustal heterogeneity. Although earlier workers<sup>16,17</sup> have suggested that intracrustal melting would produce anorthosites, this proposal differs in specifying an Al- and Eu-rich lower crust residual from the melting episodes which produced a granodioritic upper crust.

We propose that melting of Al-rich residual lower crustal material in the Proterozoic will produce dry plagioclase-rich melts, enriched in Eu, with low Rb/Sr ratios, unsupported  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios (inherited from the precursor history) and variable Sm/Nd (and  $\epsilon_{\text{Nd}}$ ) reflecting previous complex crustal history. This typically, but not exclusively, consists of (1) mantle derivation in the late Archaean; (2) extraction of granitic melts by intracrustal melting within 100–200 Myr; (3) residence time in the lower crust in a low Rb/Sr, high Sm/Nd environment for several hundred million years; and (4) a massive melting episode producing the anorthosite magma, which rises and intrudes the upper crust at ~1,500 Myr.

Ashwal and Wooden<sup>8</sup> attributed the  $\epsilon_{\text{Nd}}$  range between -6.1 and +4.1 for Proterozoic anorthosites from the Nain and Grenville Provinces to derivation from distinct mantle source regions, one enriched (Nain Province) and the other depleted (Grenville Province) in light REE (LREE). A lower crustal origin, however, is more compatible with these data. This is illustrated in Fig. 2 where the evolution of  $\epsilon_{\text{Nd}}$  values with time is shown for the upper, lower and bulk crustal compositions given in ref. 12. In 500 Myr,  $\epsilon_{\text{Nd}}$  of the lower crust decreases by two units, while the upper crust decreases by six units. Thus material can reside in the lower crust for extended periods (500 Myr) with only slight decreases in  $\epsilon_{\text{Nd}}$ , even if a period of 100–200 Myr is allowed following mantle extraction before intra-crustal differentiation occurs. Thus the Nain Province anorthosites considered by Ashwal and Wooden<sup>8</sup> to come from enriched mantle may instead represent melts of older (500 Myr) lower crustal precursors. We note that the Adirondack anorthosites which have highly positive  $\epsilon_{\text{Nd}}$  (+4) imply derivation from sources more highly depleted in LREE, or from regions with a shorter (<300 Myr) lower crustal residence time. This correlation

between crustal provinces and anorthosite Nd isotopic characteristics is consistent with our model, but becomes coincidental if anorthosites are derived from the mantle.

The initial  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of anorthosites do not directly constrain the crustal prehistory, as anorthosites are characterized by exceedingly low Rb/Sr ratios. The typical  $^{87}\text{Rb}/^{86}\text{Sr}$  ratio (0.03) resembles that of MORB sources and hence the evolution of  $^{87}\text{Sr}/^{86}\text{Sr}$  in the anorthosite source cannot readily be distinguished from that of the mantle. However, the initial Sr ratios for many anorthosites lie in the range 0.7026–0.7066 (ref. 8). The higher values are significantly greater than that expected from a direct mantle origin. This has been attributed to interaction with metasedimentary material or metamorphic fluids. An equally plausible scenario is that the anorthosite protolith existed for a limited period (100–200 Myr) with higher Rb/Sr ratios ( $^{87}\text{Rb}/^{86}\text{Sr} \approx 1$ ). In our model, this represents the period of crustal residence following mantle extraction, but before intra-crustal differentiation into a granitic upper crust and an Rb depleted lower crust.

Although this postulated sequence of events seems capable of explaining the geological, petrological, geochemical and isotopic characteristics of massif anorthosites, the source of heat required to produce massive lower crustal melting is a major problem. Two heat sources are available for melting continental crust: (1) heat released by the decay of radioactive elements within the crust; and (2) heat released from the underlying mantle and conducted through the crust. The low Rb/Sr ratio of anorthosites indicates that the parent magmas were generated from a source depleted in Rb and other incompatible elements including the heat-producing elements K, U and Th. Most concepts of crustal differentiation, including intracrustal melting and fluid migration models, similarly predict low abundances of the heat producing elements in the lower crust. We suggest that these elements were effectively removed by earlier intracrustal melting episodes which produced the granitic upper crust, and concentrated the bulk of the crustal complement of K, U and Th in the upper 10 km of the continental crust.

Accordingly, it is necessary to invoke a mantle source of heat to generate the anorthositic magmas. Such a heat source must be extensive, linear (to account for the observed geological distribution<sup>3</sup>), operate on a time scale of up to several hundred million years and be capable of heating the base of the crust to temperatures in excess of 1,300 °C. The temperature of basaltic magmas extruded at the surface of the Earth rarely exceeds 1,250 °C, but it has been shown<sup>18,19</sup> that volcanic magmas occupy a density minimum in a tholeiitic fractionation trend with the suggestion that both the crust and crustal magma chambers act as a density filter, preventing dense, primitive magmas from reaching the surface. Parent magmas for mid-ocean ridge basalts (MORB) probably had MgO contents of ~18% (refs 20–22), and liquidus temperatures around 1,400 °C (ref. 23). If a continent migrates over a mantle line plume of the mid-ocean ridge type, then temperatures in excess of 1,400 °C are likely for the upper mantle adjacent to the base of the crust. If such a plume persisted for an extended period of time, as required by our model, temperatures in excess of 1,300 °C do not seem unreasonable for the base of the crust.

Our assessment of the available data reveals that a lower crustal origin for massif-type anorthosites is plausible. On the other hand, if anorthosites can be shown to be derived from mantle sources, then these must have many of the isotopic and chemical characteristics of our lower crustal models. This implies that the magnitude of mantle isotopic and chemical heterogeneity is very much greater than presently recognized.

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## Role of paternal and maternal genomes in mouse development

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There has been much speculation on whether mammalian eggs with two male pronuclei can develop normally. Eggs with two female pronuclei can sometimes develop as far as the 25-somite stage<sup>1–3</sup> but with only very meagre extraembryonic tissues<sup>2,3</sup>. We suggested that the genome undergoes specific imprinting during gametogenesis<sup>3</sup> and that some paternal genes may be necessary for normal development of the extraembryonic tissues<sup>3,4</sup>, in which only the maternal X chromosome remains active<sup>5–9</sup>. However, the need for the maternal genome for development to term is not yet unequivocally established. The detailed study described here demonstrates that while between 40 and 50% of heterozygous reconstituted eggs with a male and a female pronucleus develop to term, none of the eggs with two male pronuclei does so. Furthermore, embryos in the latter case are very retarded, even though the trophoblast develops relatively well compared with embryos having two female pronuclei<sup>1–3</sup>. Our combined results indicate that while the paternal genome is essential for the normal development of extraembryonic tissues, the maternal genome may be essential for some stages of embryogenesis.

Fertilized eggs were obtained from (C57BL/6J × CBA/Ca) F<sub>1</sub> females (henceforth called F<sub>1</sub>) mated with MF1 albino outbred males or from MF1 ♀ × F<sub>1</sub> ♂ pairings. The female pronucleus was removed from the recipient eggs (usually from F<sub>1</sub> ♀ × MF1 ♂) and replaced with a male pronucleus from MF1 ♀ × F<sub>1</sub> ♂ to yield MF1 ♂ + F<sub>1</sub> ♂ androgenetic eggs. In a small series, F<sub>1</sub> ♂ + F<sub>1</sub> ♂ (MF1 recipient egg) or MF1 ♂ + MF1 ♂ (F<sub>1</sub> donor and recipient eggs) were also prepared. As a control for these experiments, the male MF1 pronucleus from F<sub>1</sub> ♀ × MF1 ♂ eggs was removed and replaced with another F<sub>1</sub> male pronucleus from MF1 ♀ × F<sub>1</sub> ♂ eggs, or the female F<sub>1</sub> pronucleus was removed from F<sub>1</sub> ♀ × MF1 ♂ eggs and replaced with another MF1 female pronucleus from MF1 ♀ × F<sub>1</sub> ♂ eggs (see Table 1). All the reconstituted eggs with two pronuclei were cultured overnight, when the majority of them cleaved to the two-cell stage. These embryos were transferred to the right oviducts and the control MF1 × MF1 eggs (except in the case of the MF1 ♂ + MF1 ♂ experimental eggs, when F<sub>1</sub> ♀ × F<sub>1</sub> ♂ eggs served as controls) to the left oviducts of day 1 pseudopregnant mice (day 1 = day of vaginal plug).

The results (Table 1) demonstrate that 28 androgenetic eggs implanted (23%), and eight of these contained very retarded embryos (4–6 somites) or embryonic remains when examined on day 10 or 11 of pregnancy. All the embryos showed substantially better development of extraembryonic membranes and trophoblast compared with the embryo itself (see Fig. 1 for a description of all the embryos obtained). Poor development of androgenetic embryos cannot be attributed to manipulation of the egg because, in the first series of experiments, three reconstituted eggs out of five containing a male and female pronucleus, developed normally to produce embryos with between 25 and

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**Table 1** Development of reconstituted mouse eggs

| Eggs                                                         |                  |                   | No. transferred to recipients that became pregnant/<br>total transferred | Implanted | With embryos‡  |
|--------------------------------------------------------------|------------------|-------------------|--------------------------------------------------------------------------|-----------|----------------|
| Recipient                                                    | Donor            | Reconstituted†    |                                                                          |           |                |
| (1) $F_1 \times MF1^*$                                       | $MF1 \times F_1$ | $Ak MF1 + Ak F_1$ | 90/157                                                                   | 20        | 6              |
| (2) $F_1 \times MF1$                                         | $F_1 \times MF1$ | $Ak MF1 + Ak MF1$ | 18/24                                                                    | 5         | 2              |
| (3) $MF1 \times F_1$                                         | $F_1 \times MF1$ | $Ak F_1 + Ak MF1$ | 11/17                                                                    | 1         | 0              |
| (4) $MF1 \times F_1$                                         | $MF1 \times F_1$ | $Ak F_1 + Ak F_1$ | 3/10                                                                     | 2         | 0              |
| Total no. of androgenetic eggs                               |                  |                   | 122/208                                                                  | 28        | 8 Retarded     |
| (5) $F_1 \times MF1$                                         | $MF1 \times F_1$ | $Gk F_1 + Ak F_1$ | 75/99                                                                    | 46        | 35 Live young§ |
| (6) $F_1 \times MF1$                                         | $MF1 \times F_1$ | $Ak MF1 + Gk MF1$ | 71/95                                                                    | —         | 27 Live young  |
| Total no. of control eggs with a male and female pronucleus  |                  |                   | 146/194                                                                  | —         | 62 Live young  |
| (7) Unoperated control $MF1 \times MF1$ and $F_1 \times F_1$ |                  |                   | 190/245                                                                  | 112       | 102 Normal     |

Fertilized eggs,  $F_1 \text{♀} \times MF1 \text{♂}$  and  $MF1 \text{♀} \times F_1 \text{♂}$ , were recovered 16–21 h after an injection of 5 IU human chorionic gonadotropin (HCG) in animals which had been injected previously with 5 IU pregnant mare's serum (48 h before injection of HCG) to induce superovulation. The cumulus cells were dispersed by incubation in 300 IU ml<sup>-1</sup> hyaluronidase (ovine testis; Sigma) in phosphate-buffered medium (PBI)<sup>20</sup> + 0.4% bovine serum albumin (BSA) for 3–5 min at 37 °C. Eggs were then washed 6–9 times in M16<sup>21</sup> + 0.4% BSA and cultured in this medium under paraffin oil at 37 °C in humidified 5% CO<sub>2</sub> in air. Micromanipulation of eggs was done on a Leitz micromanipulator as described previously<sup>2</sup>. The donor and recipient eggs (10–15 of each) were placed in PBI + 0.1% BSA containing the cytoskeletal inhibitors cytochalasin D and Nocodazole (Sigma) at a final concentration of 1 µg ml<sup>-1</sup> and 0.05 µg ml<sup>-1</sup> respectively, dissolved in 1.5 µl dimethyl sulphoxide<sup>2</sup>. After 30–45 min at 37 °C, the eggs were suspended in hanging drops for micromanipulation. One of the pronuclei was first removed from all the recipient eggs with a 25 µm bevelled needle using the technique described by McGrath and Solter<sup>22</sup>, to prepare haploid recipient eggs. Using the same needle, a pronucleus from donor eggs was drawn into the needle and the membrane pinched off to make a small karyoplast fragment<sup>22</sup>, followed by approximately 6 pl of β-propiolactone-inactivated Sendai virus<sup>23,24</sup> (3,000 haemagglutination units ml<sup>-1</sup>) to induce fusion<sup>25</sup>. Virus and karyoplast were then gently released into the perivitelline space underneath the zona pellucida of the recipient egg<sup>22</sup>. The procedure was repeated for each recipient egg. The recipient eggs with adhering karyoplasts were washed twice in PBI + 0.1% BSA and cultured in M16 + 0.4% BSA at 37 °C as described above. The eggs were checked for fusion of the karyoplast ~4 h after the completion of manipulation on the last group of eggs. Fusion was confirmed by the disappearance of the karyoplast and the appearance of two pronuclei within the recipient egg. The fusion rate was 80–100%. The diploid reconstituted eggs were separated, washed nine times in equilibrated M16 + 0.4% BSA and cultured overnight in this medium at 37 °C in 5% CO<sub>2</sub> in air under paraffin oil. The following morning, 98% of the eggs had cleaved and these were transferred to the oviducts of day 1 MF1, CFLP or  $F_1$  pseudopregnant mice which were obtained by mating with vasectomized males of proven sterility.

\* The  $F_1$  animals are non-albino homozygous for glucose phosphate isomerase, GPI-*bb*, while the MF1 are outbred albino, GPI-*aa*.

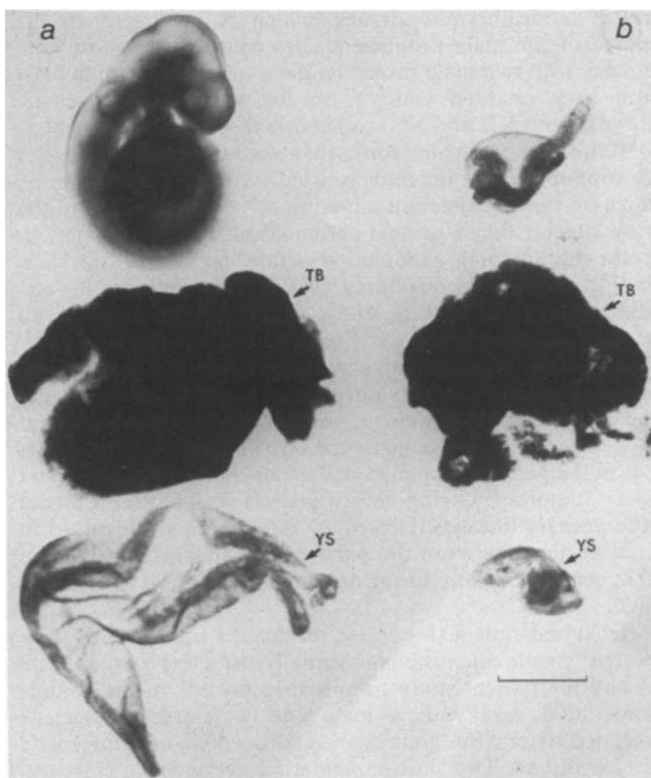
† Ak, androgenetic; Gk, gynogenetic.

‡ Androgenetic embryos were examined on day 10 of pregnancy (groups 1–4) while the reconstituted eggs with a male and a female pronucleus were allowed to proceed to day 19 (group 5) when live young were obtained by autopsy. The embryos were typed for GPI<sup>26</sup> and confirmed as GPI-*ab* (group 1), GPI-*aa* (group 2), GPI-*bb* (group 5) and GPI-*aa* (group 6).

§ Twenty-two females and 13 males were obtained by autopsy on day 19 of pregnancy.

|| Thirteen females and 14 males were obtained by natural birth. All live young from groups 5 and 6 have reached adulthood and bred normally.

**Fig. 1** Normal control embryo (a) and androgenetic embryo (b) from the same recipient on day 10 of pregnancy, each with its trophoblast (TB) and yolk sac (YS). The retarded androgenetic embryo has ~5 somites and is well formed but rather small for its stage; the trophoblast apparently is normal. The control embryo is at the 25-somite stage (scale bar 1.0 mm). Of the androgenetic embryos transferred, 23% implanted and a quarter of the decidua contained embryos or embryonic tissue at day 10. The decidua were uniformly smaller than those of the concurrent normal controls, whether or not embryos were present. Where embryos were present there was a substantial amount of trophoblastic tissue (giant cells and ectoplacental cone), and the yolk sac was substantial and expanded considering that the embryos were very retarded, unlike embryos from eggs with two female pronuclei<sup>1–3</sup>. Of the embryos present, four had reached the 4–6-somite stage (one of these was examined on day 11 and showed no signs of further development; tissue thickening and the onset of deterioration was evident). The fifth embryo had no somites but was rather large for a headfold embryo. A sixth embryo consisted of substantial but poorly organized neural tissue and somites, and a seventh had ~10 somites from the beating heart downwards, but had no head. One deciduum contained a tiny yolk sac vesicle inside a solid lump of trophoblastic tissue. There were no detectable embryonic remains in the rest of the 20 decidua. The three reconstituted control embryos analysed on day 10 were 25–35-somite embryos of normal size and in normal size decidua, indistinguishable from unoperated controls.



35 somites. The remainder of these normal reconstituted eggs were allowed to proceed to term, when 62 live young (43%) were obtained on day 19 of pregnancy (Table 1).

This study demonstrates that heterozygous androgenetic embryos are unlikely to develop to term. Three different types of androgenetic embryos would be expected, nearly 25% of them containing two Y chromosomes and dying within a few cleavage divisions<sup>8</sup>, 25% XX, and 50% XY. It is unknown which of the last two categories of embryos develops better. The maternal X chromosome is preferentially active in the extraembryonic tissue whereas X-inactivation is random in the embryonic ectoderm from which the fetus develops<sup>5-7</sup>; this may partly influence development of androgenetic embryos. In our previous study, homozygous androgenetic embryos (which are YY or XX) had an exceptionally low implantation rate (6%) and no embryonic derivatives were detected<sup>2</sup>. Our results contrast with the reported birth of two homozygous androgenetic females<sup>9</sup>, but this and similar findings<sup>9,10</sup> have not yet been substantiated<sup>2,3,11-13</sup>. The embryos with two male pronuclei in this study differ from those with two female pronuclei<sup>2,3</sup>, especially with respect to better development of the trophoblast in the former but a better embryo in the latter. There is, however, no consistent development of the experimental embryos. This could be partly due to the necessary interactions between embryonic and extraembryonic tissues<sup>14</sup>, which may be impaired to a varying extent.

We have recently suggested that the presence of a male and a female pronucleus is usually necessary for development to term and that this is probably because the paternal and the maternal genomes undergo specific imprinting as a result of epigenetic influences during gametogenesis<sup>3</sup>. There is some evidence to suggest that the differences between the paternal and the maternal genomes are recognized and may be used in embryogenesis. The nonrandom activity of the maternal X chromosome in the extraembryonic tissues is an example of such recognition<sup>5-7</sup>. Furthermore, specific paternal genes may also be necessary for the normal development of the trophoblast, as shown by studies on DDK mutant mice<sup>4</sup>. The absence of a paternal genome in an egg does result in the poor development of extraembryonic tissues<sup>2,3</sup>, for example, both triploid and dygynic embryos develop poorly but the latter in addition have meagre extraembryonic tissues, which is attributed to the removal of the male pronucleus<sup>2</sup>. By contrast, we show here that eggs with two male pronuclei have substantial trophoblast but a very retarded embryo. In the human, androgenetic embryos (both XX and XY) sometimes display development as hydatidiform moles which consist of abundant chorionic vesicles and trophoblast, but the fetus is usually absent<sup>15</sup>. Furthermore, studies on the *T<sup>hp</sup>*-deletion mutation of chromosome 17 in the mouse suggest that a normal chromosome 17 in the maternal, but not the paternal, genome is essential for normal development<sup>16-18</sup>. Hence, reconstituted eggs lacking entirely the maternal genome, as in this study, would not be expected to complete embryogenesis.

Our studies suggest that eggs with either the paternal or maternal genomes can only develop up to the blastocyst stage; some of these embryos implant and develop to a varying extent partly depending on the genotype. We suggest that the differences in the paternal and maternal genomes are recognized and may be important during embryogenesis in the establishment of the primary lineages. How these differences are involved in the interactions between the paternal and maternal genomes<sup>12</sup> and in gene expression during development remains to be determined.

After submission of this paper, results of a similar study were reported<sup>19</sup>, with conclusions essentially the same as ours (refs 2, 3 and the present study); approximately 5% of the control reconstituted eggs with a male and a female pronucleus developed to term but none of the androgenetic or gynogenetic embryos did so. Two post-implantation gynogenetic embryos, one of them with 11 somites, were detected but no androgenetic embryos were obtained.

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## A biological consequence of variation in the site of *D-J<sub>H</sub>* gene rearrangement

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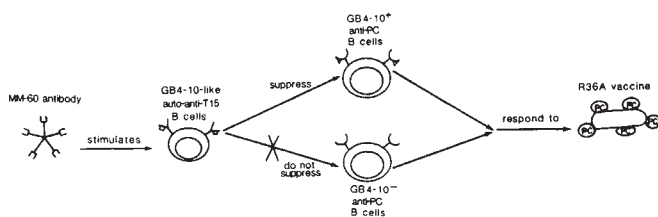
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One mechanism which generates diversity in immunoglobulin variable (V) regions is flexibility in the site of recombination among the constituent genetic elements<sup>1-7</sup>. Within a specific antibody family (that is, a particular *V<sub>H</sub>-V<sub>L</sub>* combination), variability in *V-D-J* rearrangement not only leads to sequence diversity at the boundary of the juxtaposed genes, but also enables the total length of the third complementarity-determining region (CDR-3) of the heavy chain to be conserved<sup>8</sup>. We demonstrate here that the junctional diversity inherent in rearranged immunoglobulin genes can have consequences for the biology of the immune system. Sequence analysis of the expressed immunoglobulin genes of idiotypically variant as opposed to conventional B lymphocytes of a dominant antibody family showed that the variant B cells undergo a novel *D-J<sub>H</sub>* joining event such that an extra amino acid is inserted into the heavy chain CDR-3. The unique D-region conformation possessed by the variant B cells accounts for previous observations which showed that variant and conventional B cells could be differentially regulated *in vivo* by an autologous set of idiotope-specific B lymphocytes<sup>9</sup>. Our findings indicate that D-region structure can determine the expression of regulatory idiotypes and suggest that the conservation of heavy-chain CDR-3 length within an antibody family may reflect regulatory as well as functional constraints.

Serological studies using monoclonal anti-idiotypic antibodies and sequence analysis of monoclonal immunoglobulins have shown that there is a significant degree of structural diversity among BALB/c anti-phosphorylcholine (anti-PC) antibodies belonging to the dominant T15 idiotype antibody family<sup>10-12</sup>. Using the A/J anti-T15 monoclonal antibodies AB1-2 and GB4-10, a minor subset of T15<sup>+</sup> anti-PC IgM antibodies can be detected in BALB/c mice which do not express the latter idiotope (constituting 0-3% of the normal *in vivo* anti-PC IgM response<sup>10</sup>). The biological significance of this particular idiotypic heterogeneity became apparent when an *in vivo* B-cell counterpart to the GB4-10 anti-T15 hybridoma was found in





**Fig. 1** Schematic diagram of a monoclonal model for idiotype-directed regulation of immunoglobulin production. *In vivo* administration of the auto-anti-(anti-T15) monoclonal antibody, MM-60 (ref. 13) induces proliferation among a restricted, possibly clonal, set of auto-anti-T15 B cells possessing a specificity identical to the A/J anti-T15 hybridoma GB4-10 (ref. 9). Once stimulated, 'GB4-10-like' anti-T15 B cells suppress anti-PC IgM production from only those B cells bearing the GB4-10-defined idiotype; anti-PC IgM production by GB4-10<sup>-</sup> B cells is unimpaired. The anti-PC humoral response of BALB/c mice is dominated by the GB4-10<sup>+</sup> component, with 95–100% of all anti-PC serum IgM bearing the GB4-10-defined idiotype<sup>10</sup>. All GB4-10<sup>+</sup> anti-PC antibodies are also T15<sup>+</sup>, while 40% of GB4-10<sup>-</sup> anti-PC IgM antibodies in primary immune response are T15<sup>+</sup> (that is, the variant population studied here), the remainder belonging to the M511 and M603 idiotypic families<sup>10,34</sup>.

BALB/c mice and was shown to specifically suppress the production of anti-PC IgM from B cells bearing the GB4-10-defined idiotype<sup>9</sup>. Figure 1 summarizes, from previous work<sup>9,13</sup>, the cellular and humoral interactions involved in this differential regulation of the primary *in vivo* B-cell response to PC and how it approximates to a monoclonal model for autologous idiotype-directed immunoregulatory processes. Determining the structural correlate of the GB4-10-defined idiotype would indicate which region(s) of the immunoglobulin molecule are responsible for the expression of regulatory idiotypes and also what genetic changes lead to the loss of such idiotypes, thereby permitting a B lymphocyte to avoid regulation.

To achieve this, four B-cell hybridomas secreting a monoclonal anti-PC IgM of the T15<sup>+</sup> GB4-10<sup>-</sup> phenotype (designated 2-21, 5-18, 7-22 & 12-3) were identified following fusion of the nonproductive plasmacytoma Ag8.653 (ref. 14) to spleen cells from BALB/c mice undergoing a primary immune response to R36A pneumococcal vaccine. The monoclonal antibodies from

all four variant hybridomas bind PC-protein conjugates and R36A bacterial cells; the purified IgM from the variant 12-3 hybridoma appears to bind PC with an affinity comparable with that of BH8, a monoclonal T15<sup>+</sup> GB4-10<sup>+</sup> anti-PC IgM of germ-line sequence (B. A. P., unpublished observation). To prove that those hybridomas from the same fusion (2-21, 5-18, 7-22) were the immortalized products of distinct B-cell clones, the nonproductively rearranged heavy-chain gene of each hybridoma was examined by Southern blot analysis using a *J<sub>H</sub>* region-specific probe<sup>15</sup>. Each hybridoma possessed a different sized *J<sub>H</sub>*-containing *Eco*RI restriction fragment from its nonproductive allele, demonstrating that each hybridoma was derived from independent anti-PC B-cell precursors (data not shown). In addition, no gross structural differences in the T15 *V<sub>H</sub>*-*J<sub>H</sub>*1 gene complex were detected between the variant hybridomas and the prototypic T15<sup>+</sup> anti-PC plasmacytoma S107 (heavy- and light-chain genes of germ-line sequence<sup>16,17</sup>) as shown by the presence of a 7.5-kilobase (kb) *J<sub>H</sub>*-containing *Eco*RI fragment<sup>5</sup> in all cases.

Loss of expression of GB4-10-defined idiotype could be due to subtle alterations in light- and/or heavy-chain structure as both T15 *V<sub>L</sub>* and *V<sub>H</sub>* regions contribute to idiotype expressions<sup>9,10</sup>. Examination of light-chain structure by isoelectric focusing showed that purified  $\kappa$  light chains from two variant hybridomas (5-18, 7-22) co-migrated with the secreted  $\kappa$  chain of S107 (*pI* 8.0) (ref. 9). The germ-line character of the light chains produced by GB4-10<sup>-</sup> variant B cells was confirmed by sequencing portions of the  $\kappa$ -chain mRNA from the hybridomas 12-3 and 5-18 (see Fig. 2 legend); the  $\kappa$  mRNA sequence from both variant hybridomas was identical to the S107A  $\kappa$  gene<sup>17</sup> from the beginning of CDR-2 (codon 50) to *J<sub>k</sub>*1 (codon 108) (data not shown). The presence of mutations upstream of CDR-2 in the T15 *V<sub>L</sub>* gene of the variant is highly unlikely as somatic mutation in the expressed light-chain genes of IgM-producing B cells (as seen in representative hybridomas) is rare<sup>12,18,19</sup>. In support of this, experiments using recombinant immunoglobulin molecules from GB4-10<sup>+</sup> and GB4-10<sup>-</sup> T15<sup>+</sup> anti-PC hybridomas confirm that expression of the GB4-10-defined idiotype in the T15 family is associated with the heavy chain<sup>9</sup>. Hence, alterations in the T15 *V<sub>L</sub>* structure do not appear to be the basis for the absence of expression of this regulatory idiotype.

|      |     |                                                                                |          |                                               |
|------|-----|--------------------------------------------------------------------------------|----------|-----------------------------------------------|
| S107 | (+) | GCAAGTAGAAACAAAGCTAATGATTATACAACAGAGTACAGTGCATCTGTGAAGGGTCGGTTCATCGTCTCCAGAGAC | V REGION | GAGTACAGTGCATCTGTGAAGGGTCGGTTCATCGTCTCCAGAGAC |
| BH8  | (+) | GCAAGTAGAAACAAAGCTAATGATTATACAACAGAGTACAGTGCATCTGTGAAGGGTCGGTTCATCGTCTCCAGAGAC |          |                                               |
| 2-21 | (-) | GATTATACAACAGAGTACAGTGCATCTGTGAAGGGTCGGTTCATCGTCTCCAGAGAC                      |          |                                               |
| 5-18 | (-) | GCAAGTAGAAACAAAGCTAATGATTATACAACAGAGTACAGTGCATCTGTGAAGGGTCGGTTCATCGTCTCCAGAGAC |          |                                               |
| 7-22 | (-) |                                                                                |          | ACGGT-GGTT-ATCGTCTCCAGAGAC                    |
| 12-3 | (-) |                                                                                |          |                                               |

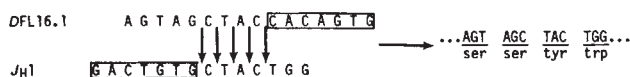
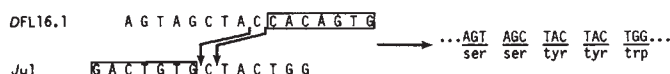
  

|                                                                               |                    |          |
|-------------------------------------------------------------------------------|--------------------|----------|
| ACTTCCCAAAGCATCCTCTACCTTCAGATGAATGCCCTGAGAGCTGAGGACACTGCCATTATTACTGTGCAAGAGAT | TACTACGGTAGTAGC    | D REGION |
| ACTTCCCAAAGCATCCTCTACCTTCAGATGAATGCCCTGAGAGCTGAGGACACTGCCATTATTACTGTGCAAGAGAT | TACTACGGTAGTAGC    |          |
| ACTTCCCAAAGCATCCTCTACCTTCAGATGAATGCCCTGAGAGCTGAGGACACTGCCATTATTACTGTGCAAGAGAT | TACTACGGTAGTAGCTAC |          |
| ACTTCCCAAAGCATCCTCTACCTTCAGATGAATGCCCTGAGAGCTGAGGACACTGCCATTATTACTGTGCAAGAGAT | TACTACGGTAGTAGCTAC |          |
| ACTTCCCAAAGCATCCTCTACCTTCAGATGAATGCCCTGAGAGCTGAGGACACTGCCATTATTACTGTGCAAGAGAT | TACTACGGTAGTAGCTAC |          |
| ACTTCCCAAAGCATCCTCTACCTTCAGATGAATGCCCTGAGAGCTGAGGACACTGCCATTATTACTGTGCAAGAGAT | TACTACGGTAGTAGCTAC |          |

|                                                      |                                            |                |
|------------------------------------------------------|--------------------------------------------|----------------|
| TACTGTTACTTTCGATGCTCTGGGGCCAGGGACACGGTCACCGTCTCTCTCA | GAGAGTCACTCCTTCCAAATGTCTTCCCCCTCGTCTCTCTGC | C $\mu$ REGION |
| TACTGTTACTTTCGATGCTCTGGGGCCAGGGACACGGTCACCGTCTCTCTCA | GAGAGTCA                                   |                |
| TACTGTTACTTTCGATGCTCTGGGGCCAGGGACACGGTCACCGTCTCTCTCA | GAGAGTCACTCTCTT                            |                |
| TACTGTTACTTTCGATGCTCTGGGGCCAGGGACACGGTCACCGTCTCTCTCA | GAGAGTCACTCTCTT                            |                |
| TACTGTTACTTTCGATGCTCTGGGGCCAGGGACACGGTCACCGTCTCTCTCA | GAGAGTCACTCTCTT                            |                |
| TACTGTTACTTTCGATGCTCTGGGGCCAGGGACACGGTCACCGTCTCTCTCA | GAGAGTCACTCTCTT                            |                |
| TACTGTTACTTTCGATGCTCTGGGGCCAGGGACACGGTCACCGTCTCTCTCA | GAGAGTCACTCTCTT                            |                |
| TACTGTTACTTTCGATGCTCTGGGGCCAGGGACACGGTCACCGTCTCTCTCA | GAGAGTCACTCTCTT                            |                |

**Fig. 2** Nucleotide sequence of the *V<sub>H</sub>*-5'*C $\mu$*  region for several idiotypically variant and conventional T15<sup>+</sup>BALB/c monoclonal anti-PC immunoglobulins. The heavy-chain sequence of the S107 plasmacytoma<sup>5</sup> serves as the prototypic sequence for BALB/c anti-PC antibodies as it expresses the germ-line T15 *V<sub>H</sub>*, DFL16.1 and *J<sub>H</sub>*1 gene sequences in rearranged form<sup>5,19</sup>. The IgM constant-region gene sequence<sup>35</sup> replaces the IgA constant region in the S107 prototypic sequence as all the immunoglobulins studied here are of the IgM class. Undetermined bases (sequence ambiguities) are denoted by dashes and reactivity with the anti-T15 idiotype antibody GB4-10 is shown as (+) or (-). Sequencing of the hybridoma heavy-chain mRNA was performed as described by Caton *et al.*<sup>36</sup>. A 17-base synthetic oligonucleotide primer complementary to the 5' end of the first exon of the  $\mu$  heavy-chain constant gene (underlined nucleotides) was used to initiate reverse transcription of poly(A)<sup>+</sup> cytoplasmic RNA. Specific dideoxynucleotides were included in the reaction mixtures to create base-specific stops during cDNA synthesis with [ $\alpha$ -<sup>32</sup>P]dATP or [ $\alpha$ -<sup>35</sup>S]dATP as the incorporated label. The nucleotide sequence of the cDNA was determined by electrophoresing the labelled cDNA fragments on polyacrylamide gels, followed by autoradiography of the gel. The first 10 bases distal to the primer were consistently indiscernible and the maximum sequence length accurately readable by this method was approximately 250 bases, which permitted sequencing of the entire CDR-2. The confirmatory heavy-chain sequence of the T15<sup>+</sup> GB4-10<sup>+</sup> anti-PC IgM-secreting BALB/c hybridoma BH8 (ref. 10) eliminates the possibility that the insertion of the TAC codon at the D-*J<sub>H</sub>* junction is an artefact of our procedure for sequencing  $\mu$  heavy chains.

GB4-10<sup>+</sup> nonvariant D-J<sub>H</sub> joiningGB4-10<sup>-</sup> variant D-J<sub>H</sub> joining

**Fig. 3** Pattern of D-J<sub>H</sub> gene rearrangement in GB4-10<sup>+</sup> versus GB4-10<sup>-</sup> anti-PC B-cell precursors. Rearrangement between the DFL16.1 and J<sub>H</sub>1 genes could occur at five and two alternate sites in GB4-10<sup>+</sup> nonvariant and GB4-10<sup>-</sup> variant anti-PC immunoglobulins, respectively (designated by arrows); the two D-J<sub>H</sub> joining positions for GB4-10<sup>-</sup> variant antibodies are distinct from all the possible sites used by the GB4-10<sup>+</sup> nonvariant group. The boxes show the conserved heptamer recognition sequences defined by Sakano *et al.*<sup>7</sup>. The J<sub>H</sub>1 and DFL16.1 gene sequences have been described previously<sup>5-7,20</sup>.

The primary structure of the heavy chain expressed by each variant and a nonvariant hybridoma was examined by sequencing the  $\mu$  heavy-chain mRNA in the same way (Fig. 2). The heavy chains of all the variant hybridomas are coded by T15 V<sub>H</sub> (ref. 6), DFL16.1 (ref. 20) and J<sub>H</sub>1 (ref. 5); the same V<sub>H</sub>, D and J<sub>H</sub> genes as the two nonvariant heavy chains. The heavy-chain gene sequences of the variants are also identical with the germ-line (that is, unmutated) sequence of the constituent T15 V<sub>H</sub>, DFL16.1 and J<sub>H</sub>1 genes (for codons 50-113). The only notable difference in heavy-chain gene structure exhibited by the variant group is the presence of an extra tyrosine codon (TAC) at the D-J<sub>H</sub> gene junction. That this structural peculiarity is the sole basis for loss of the GB4-10 regulatory idiotype is strengthened by the finding that amino acid substitutions present throughout V<sub>H</sub> of T15<sup>+</sup> anti-PC antibodies (including those in regions not sequenced here, such as in CDR-1) have no effect on idiotype expression (ref. 9 and B.P., unpublished data).

Examination of the nucleotide sequence at the 3' end of the germ-line DFL16.1 gene<sup>20</sup> indicates how this insertion might occur (Fig. 3). Although the site of D-J<sub>H</sub> joining cannot be precisely defined for either the conventional or the variant T15<sup>+</sup> antibody group, generation of the two distinct heavy chains is clearly dependent on different D-J<sub>H</sub> gene recombinations. Considering the consistency in length and composition of this site, it is highly unlikely that it derives from an 'N segment'—the non-templated, G+C-rich region often present at the D-J<sub>H</sub> boundary<sup>21</sup>. Detection of functional variants within the virgin anti-PC B-cell repertoire of neonatal mice by the clonal splenic focus assay<sup>22,23</sup> (B. A. P. and M. V., unpublished observations), is consistent with the molecular evidence showing that generation of these variant B cells is determined at an early stage of B-cell ontogeny. Although insertion of an extra codon at the site of recombination among immunoglobulin genes is not a rare occurrence, the heavy-chain gene structure of the T15<sup>+</sup> GB4-10<sup>-</sup> B cells is unusual because the insertion here is not accompanied by a compensating deletion of a codon at the V<sub>H</sub>-D junction; this results in a heavy-chain CDR-3 longer by one amino acid residue than the CDR-3 length of all other sequenced T15<sup>+</sup> antibodies.

Consistency in heavy-chain CDR-3 length is not unique to the T15 antibody family, but is an apparently universal characteristic among antibody families which dominate a particular antigen response; pauciclonal systems which exhibit this trait include the anti- $\alpha$ -1, 3 dextran<sup>24</sup>, anti-galactan<sup>25</sup>, anti-inulin<sup>26</sup>, anti-GAT<sup>27,28</sup> and anti-phenylloxazalone responses<sup>29</sup>. Although conservation of a particular CDR-3 conformation would most reasonably be based on some functional criterion such as antigen binding activity or light-chain pairing potential, this may not

necessarily be the case. Variation in CDR-3 primary structure due to utilization of different D genes and/or flexibility in V<sub>H</sub>-D-J<sub>H</sub> joining apparently alters neither the antigen specificity nor the ability to pair with the respective light chain for anti-dextran, anti-galactan and anti-PC antibodies<sup>8</sup>. In addition, consistency in CDR-3 length for conventional T15<sup>+</sup> anti-PC antibodies cannot be due to a highly preferential V-D-J recombination which precludes all others, as several anti-PC antibodies containing non-T15 light chains and heavy chains coded for by the T15 V<sub>H</sub>, DFL16.1 and J<sub>H</sub>1 genes do exhibit different CDR-3 lengths<sup>12</sup>. Our results indicate that deviation from uniformity in CDR-3 length does not necessarily affect antigen binding or light-chain pairing, but can lead to destruction of a regulatory idiotope and possibly, to the creation of a regulatory idiotope(s) unique to CDR-3 length variants. Conservation of a particular CDR-3 length within a dominant antibody family may be necessary to ensure that target idiotopes responsible for the coordinated regulation of an immune response are properly displayed to the surrounding milieu. The validity of such a model is supported by studies on tertiary structure of V regions of monoclonal anti-PC<sup>30</sup> and anti-galactan<sup>31</sup> antibodies which show that D-region residues are prominently exposed on the external surfaces of the immunoglobulin molecule. Regularity in heavy-chain CDR-3 length within an antibody family may thus reflect a B lymphocyte's niche in the idiotypic network rather than being due solely to antigen binding or light-chain pairing.

It is intriguing that most of the family-specific idiotopes defined so far on mouse immunoglobulins using mouse anti-idiotypic antibodies have been mapped to the D regions<sup>25,32,33</sup>. However, a model of idiotype-specific immunoregulation based on recognition of D-region-determined idiotopes presumably necessitates the existence of an extensive number of anti-idiotypic elements considering the degree of D-region sequence variability often present within an antibody family<sup>25</sup>. Our studies on the 'mini-network' described here suggest an alternative model; the expression of a D-region-determined regulatory idiotope can be resistant to many of the sequence substitutions in a given D-region (ref. 9 and B.P., unpublished data), with loss of idiotope expression brought about by only a few, significant perturbations in D-region tertiary structure (for example, insertion of a bulky, non-polar amino acid at the D-J<sub>H</sub> junction). Examination of other autologous idiotype-specific immunoregulatory systems which function *in vivo* should help to resolve whether expression of regulatory idiotopes are generally determined by D-region/CDR-3 conformation.

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## Major surface antigen gene of a human malaria parasite cloned and expressed in bacteria

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The late blood stages of the human malaria parasite, *Plasmodium falciparum*, carry a major surface antigen, p190, of molecular weight ( $M_r$ ) 190,000 (ref. 1). This antigenically variable protein<sup>1–3</sup> is actively processed, first as the parasite matures and again when it is released into the blood stream and invades a new erythrocyte to initiate a cycle of growth<sup>1</sup>. It elicits a strong immune response in man; all tested adult sera from endemic areas have antibodies against this protein<sup>1</sup>. Our evidence indicates that purified p190 can alter the course of parasitaemia in monkeys with falciparum malaria. We have also succeeded in cloning part of the gene for p190 and expressing it in *Escherichia coli*. To this end we have developed a new technique, antibody select, which greatly simplifies final identification of expressing clones.

The protein p190 was first defined<sup>2</sup> and later purified<sup>1</sup> by monoclonal antibodies. It begins to be made in schizogony as a 190,000  $M_r$  protein. Processing creates smaller peptides, notably major species of approximately 160,000, 125,000, 106,000 and 82,000  $M_r$  (ref. 1 and R.H., unpublished). These and other features strongly suggest that p190 is related to the 195,000  $M_r$  protein studied by Holder and Freeman<sup>4</sup>, and to similar proteins in rodent<sup>5–7</sup> and simian<sup>8,9</sup> parasites. One part of p190, defined by monoclonal antibody 2.2, is present in all isolates of *P. falciparum* tested; another part, defined by antibody 7.3, is present in p190 purified from the Thai isolate K1 (ref. 10) but absent from some other isolates<sup>2,3</sup>. Genetically pure lines from a single Thai patient vary in this part of the protein<sup>11</sup>. The antigenically variable part of the protein is cleaved off during release and re-invasion, whilst the constant part survives into the next cycle<sup>1</sup>.

Preliminary studies suggest that p190 can protect monkeys against falciparum malaria. Immunization with the affinity-purified native protein modifies the course of infection by the parasite. We treated four control monkeys (Fig. 1) with Freund's complete adjuvant for the first immunization only and with incomplete adjuvant for subsequent immunizations before challenge with the parasite. All show a rapid rise in parasitaemia to the level (20%) at which drug treatment is given to prevent death of the animals. By contrast, two of the three immunized monkeys after an initial rise, rapidly lose detectable parasites and recover. All the immunized monkeys develop antibodies against p190 and show an anamnestic response after challenge (Table 1). Interestingly, the variable part of the p190 protein

Table 1 Antibody response to p190 immunization

| Monkey | Inoculated with: | Antibodies against p190 (reciprocal titres) |        |         |
|--------|------------------|---------------------------------------------|--------|---------|
|        |                  | a                                           | b      | c       |
| Sven   | FCA              | <100                                        | <100   | 100     |
| Uli    | FCA              | <100                                        | <100   | 100     |
| Dora   | FCA              | <100                                        | <100   | 900     |
| Ronnie | FCA              | <100                                        | 100    | 100     |
| Laura  | p190 + FCA       | <100                                        | 24,300 | >72,900 |
| Anita  | p190 + FCA       | 100                                         | 8,100  | >72,900 |
| Hugo   | p190 + FCA       | <100                                        | 24,300 | >72,900 |

Anti-p190 antibodies assayed by ELISA in the treated monkeys. a, Before immunization; b, after immunization; c, 30 days after challenge. FCA, Freund's complete adjuvant. Microtitre plates (Nunc) were coated with affinity-purified p190: 50  $\mu$ l containing ~100 ng were added to each well and incubated (room temperature; overnight), washed thoroughly ( $\times 5$ ) with tap water. Remaining sites were blocked with 100  $\mu$ l of bovine serum albumin (BSA) solution (3% in phosphate-buffered saline, PBS) for 30 min (room temperature). Threefold dilutions (50  $\mu$ l) of monkey sera (in 1% BSA in PBS) were incubated (room temperature; 6 h) in each well; the plate was washed (tap water) before adding urease-conjugated rabbit anti-Saimiri IgG antiserum (50  $\mu$ l of an appropriate dilution in 1% BSA (as above), overnight at room temperature and washed again ( $\times 4$  in tap water  $\times 2$  in distilled water). 150  $\mu$ l of urease substrate (urea, 0.1%; 0.008% bromocresol purple; 0.2 mM EDTA; pH 4.8) was added to each well and incubated (37 °C, 45 min). A positive reaction registers as a change from purple to yellow<sup>27</sup>.

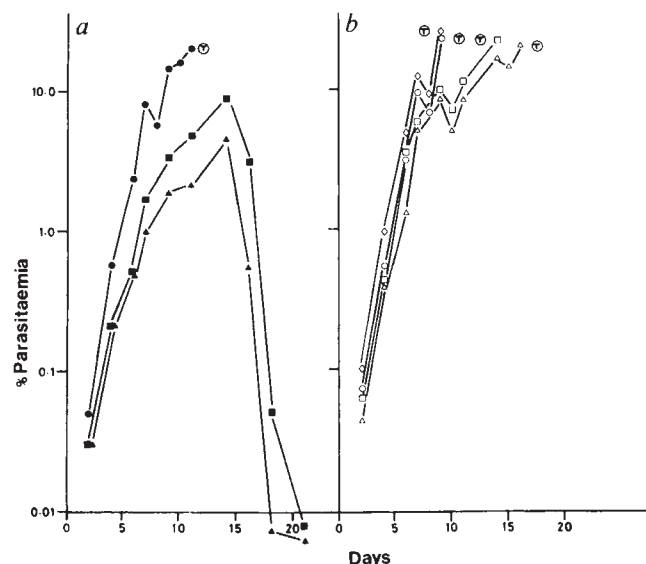


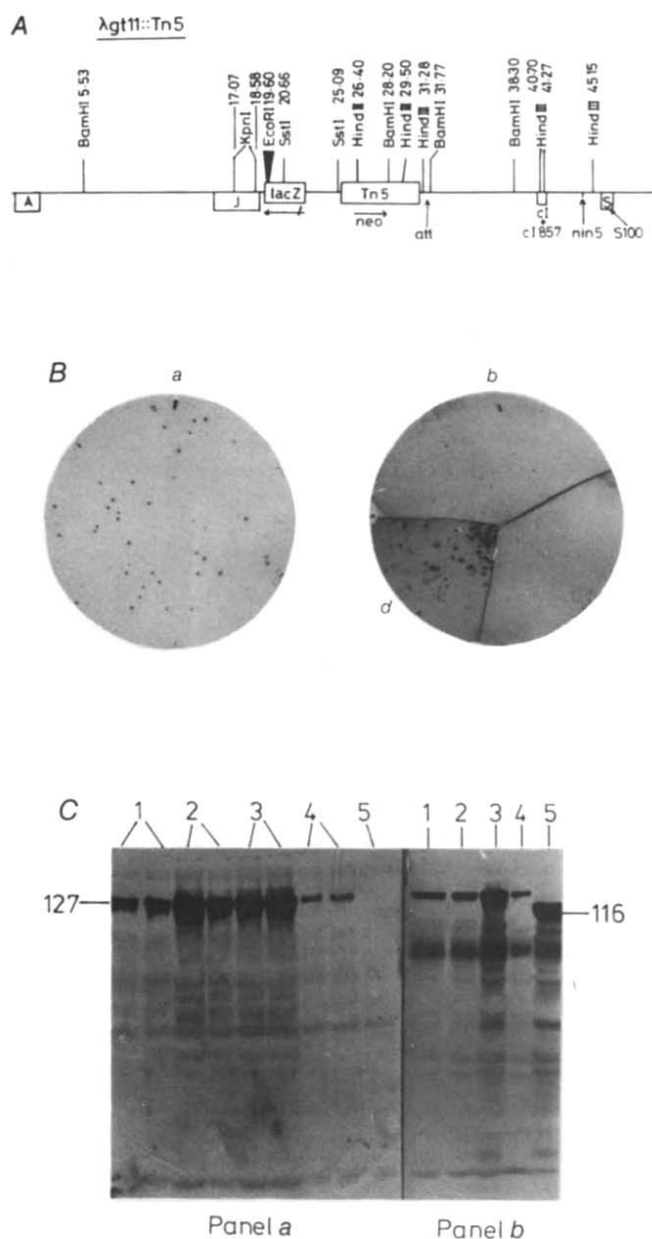
Fig. 1 Effect of p190 on the course of parasitaemia in Saimiri monkeys. a, Immunized monkeys. Affinity-purified p190 was injected into three monkeys with Freund's complete adjuvant—Anita (●), Hugo (■) and Laura (▲). Each received subcutaneously 50  $\mu$ g at day -52, 50  $\mu$ g at day -39 and 50  $\mu$ g at day -19. b, Controls (open symbols) received adjuvant alone. After immunization, all the monkeys were challenged intravenously with  $\sim 2 \times 10^7$  parasitized erythrocytes (*P. falciparum*, Palo Alto strain) from a single donor monkey. ⊕ indicates drug treatment. Parasitaemias were estimated by Giemsa-stained smears.

used in this experiment (purified from isolate K1), was different from its homologue in the challenge parasite (Palo Alto strain)<sup>2,3</sup>. This suggests that the constant part of p190 may have protective value.

Much could be learned about this protein from cloning its gene. In addition, clones expressing the gene in bacteria would be a ready source of a protein which is a strong candidate for a subunit vaccine against falciparum malaria. Recently, DNA sequences encoding antigens from both *P. knowlesi*<sup>12</sup> and *P. falciparum*<sup>13</sup> have been cloned. These studies showed that clones

**Fig. 2** Isolation of cDNA clones containing p190 sequences. **A**, Cloning vector. Phage  $\lambda$ gt11::Tn5 was isolated following transposition of Tn5 to  $\lambda$ gt11<sup>14</sup>. The cDNA is inserted at the unique *Eco*RI site in *lacZ*, positions of important restriction sites are indicated in kb. The phage is temperature-inducible (*cl*<sub>857</sub>) and carries the *nin5* deletion and an amber mutation (S100) preventing lysis in the absence of a suppressor. The presence of Tn5 greatly simplifies isolation of lysogens, as these derivatives are resistant to kanamycin. **B**, Colony screening of the  $\lambda$ gt11::Tn5 cDNA expression library by ELISA. **a**, Positive colonies from the primary screen probed with anti-p190 serum. **b**, p190 negative colonies from the primary screen probed with anti-p190 serum. **c**, Positive colonies from the primary screen probed with non-immune rabbit serum. **d**, p190-negative colonies from the primary screen probed with anti- $\beta$ -galactosidase serum. **C**, Western blots of total protein from four p190 lysogens probed with anti-p190 serum (panel **a**, lanes 1–4, each clone run in duplicate) and anti- $\beta$ -galactosidase serum (panel **b**, lanes 1–4). Lane 5 in each panel shows a control lysogen carrying the vector with no cDNA insert.

**Methods:** The  $\lambda$ gt11::Tn5 cDNA library in BTA282,  $\Delta$ lacU<sub>169</sub> $\Delta$ lon *araD139 str<sup>R</sup> thi hfl A150* (chr::Tn10) *hsdR<sup>-</sup>M<sup>+</sup>* (a derivative of RY1083, see ref. 14) was grown overnight at 30 °C on L-agar with 1555  $\mu$ g ml<sup>-1</sup> tetracycline and 40  $\mu$ g ml<sup>-1</sup> kanamycin. Colonies were replicated onto nitrocellulose and incubated 2 h at 30 °C. Phage were induced by incubation at 42 °C for 90 min. Colonies were lysed by a modification of the method of Stanley<sup>28</sup>. Nitrocellulose disks were placed on a pad soaked in 5% SDS and heated in a 100 °C oven for 15 min. They were immersed (1 h, 20 °C) in 5% ovalbumin in 10 mM Tris-Cl, pH 8.0, 150 mM NaCl (5% OA/TS) containing 0.01% NaN<sub>3</sub>, 0.2 mg ml<sup>-1</sup> henylmethylsulphonyl fluoride (PMSF) and 10  $\mu$ g ml<sup>-1</sup> DNase I, washed twice in TS and electroeluted (1 h, 50 V) to remove SDS. The filters were further blocked in 5% OA/TS (15 min, 20 °C) and incubated overnight in rabbit anti-p190 serum diluted 1:150 in 5% OA/TS (5 ml). They were washed 5 $\times$  in TS and incubated in 1:1,000 dilution of peroxidase-conjugated goat anti-rabbit IgG in 5% OA/TS (4 h, 20 °C). After a further five washes in TS, the ELISA reaction was developed<sup>29</sup>. The anti-p190 serum was raised in a New Zealand White rabbit against affinity-purified p190 protein<sup>1</sup>. It was extensively preadsorbed on induced  $\lambda$ gt11::Tn5 lysogens of BTA282 to remove nonspecific anti-*E. coli* antibodies. Total proteins from lysogens for gel analysis were obtained as follows: Young L-broth cultures of lysogens (in BTA282), containing 15  $\mu$ g ml<sup>-1</sup> tetracycline and 40  $\mu$ g ml<sup>-1</sup> kanamycin, were induced (90 min, 42 °C), centrifuged (1,500g, 10 min) and the bacteria ( $\sim 2 \times 10^9$ ) resuspended in 60  $\mu$ l of 10% SDS, 0.1 mg ml<sup>-1</sup> PMSF (15 min, 20 °C) and boiled (2 min). 180  $\mu$ l of Laemmli<sup>30</sup> sample buffer containing 7% 2-mercaptoethanol was added and after further boiling, the samples were electrophoresed on 7.5% SDS-polyacrylamide gels and Western blotted essentially as described previously<sup>29,31</sup>. The filters were probed with antibody as above. Markers used to calibrate the gels were ferritin (220,000 *M<sub>r</sub>*), phosphorolase *b* (94,000 *M<sub>r</sub>*), bovine serum albumin (69,000), catalase (60,000), ovalbumin (46,000), lactate dehydrogenase (36,000) and carbonic anhydrase (30,000).



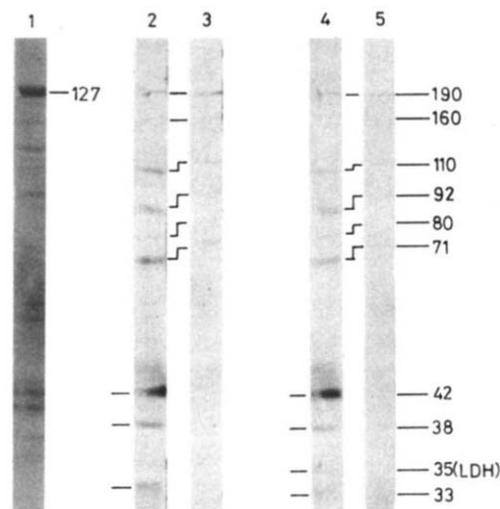
can be detected using antibody probes to screen bacteria expressing parts of the parasite protein. We report here isolation of a p190 cDNA clone detected by a similar strategy. It includes the 3' terminus of the messenger RNA and the sequence encoding the final 70 aminoacids at the C-terminus of the protein.

We have made a useful derivative of phage  $\lambda$ gt11 to serve as the cloning vector for cDNA from cultured *P. falciparum* blood stages. Like its parent,<sup>14,15</sup> our derivative ( $\lambda$ gt11::Tn5) facilitates expression of cloned cDNA in *E. coli*. The transposon Tn5 confers kanamycin resistance on lysogens, which can thus be very easily selected (M.G., N. Bone and J.S., unpublished results). The cDNA was made from total poly(A)<sup>+</sup> RNA of blood stage *P. falciparum* isolate KI, ligated to *Eco*RI linkers and cloned<sup>16</sup> into the unique *Eco*RI site of the vector. The cDNA is thereby inserted into the *lacZ* gene, encoding  $\beta$ -galactosidase (Fig. 2A). In the correct frame and orientation at this site, a cDNA molecule is expressed as a hybrid protein comprising most of the  $\beta$ -galactosidase molecule fused to a *P. falciparum* polypeptide (the *Eco*RI site is 153 base pairs (bp) from the C-terminus of the gene)<sup>14,17</sup>.

Recombinant phages ( $\sim 7,000$ ) were screened for synthesis of p190 peptides. Small colonies lysogenic for the phages were transferred to nitrocellulose, induced to stimulate fusion protein synthesis and probed (see Fig. 2) with a polyclonal rabbit antiserum raised against affinity-purified p190<sup>1</sup>. Positive candidate clones were detected by enzyme-linked immunosorbent assay (ELISA) with horseradish peroxidase-conjugated goat anti-rabbit IgG antiserum (Fig. 2B). Four apparently identical clones were obtained from this screening. They each encode a hybrid protein (Fig. 2C) significantly larger (127,000 *M<sub>r</sub>*) than native  $\beta$ -galactosidase<sup>15</sup>. In Western blots (Fig. 2C) they can be seen to have epitopes recognized by both the anti- $\beta$ -galactosidase (right panel) and the anti-p190 (left panel) probes. By contrast, control clones synthesizing native enzyme only respond to the anti- $\beta$ -galactosidase probe.

To confirm that our clones encode a p190 sequence, we have developed a rapid and simple technique which we call antibody select. This method eliminates false positive clones which could register in the initial screening if, for example, the anti-p190 probe contained antibodies against other *P. falciparum* pro-





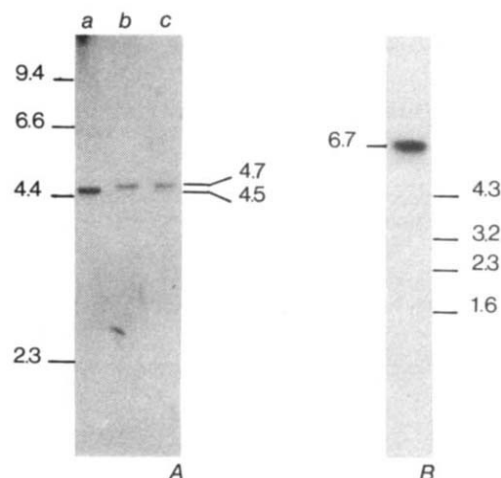
**Fig. 3** Confirmation of a  $\lambda$ p190 clone by antibody select. Lane 1, p190 hybrid protein used in antibody select (see lanes 3, 5). The protein isolated from an induced lysogen (see below) and run on an SDS-polyacrylamide gel was stained with Coomassie blue. The major band is at 127,000  $M_r$  (see Fig. 2C). Lanes 2–5, Western blots of total KI parasite proteins using the following probes: lane 2, whole anti-p190 serum; lane 3, immunoglobulins affinity-purified from the whole serum by the hybrid protein; lane 4, whole serum with added rabbit antibody against parasite lactate dehydrogenase (LDH); lane 5, immunoglobulins affinity-purified from the mixed sera of lane 4 by the hybrid protein.

**Methods:** p190 hybrid protein for antibody select was purified as follows: Approximately  $10^{11}$  lysogens (in BTA282) harbouring p190-1 were grown up and collected as described in Fig. 2 legend. The bacterial pellet was resuspended in 4 ml cold 10 mM Tris-Cl, pH 8.0, 100 mM NaCl, 1 mM EDTA (TNE) and left on ice 5 min. 4 ml of 50% sucrose in TNE were added, followed 10 min later by 8 ml of 4 mg ml<sup>-1</sup> lysozyme. After a further 15 min on ice, 4 ml of 0.25 M EDTA, pH 8.0 and 2 ml of 10% Nonidet P-40 were added. The suspension was freeze-thawed and the DNA sheared by 5 $\times$  extrusion through a 19-gauge needle. The extract was then spun at 13,000g, 4 $^{\circ}$ C, 30 min and the pellet suspended in 15 ml of 8 M urea. After a further spin (13,000g, 4 $^{\circ}$ C, 20 min), the supernatant, which contained purified fusion protein, was retained. For antibody select, 4 ml of this supernatant was incubated with 5 cm<sup>2</sup> of nitrocellulose (2 h, 20 $^{\circ}$ C). After washing and blocking the filter as described for Fig. 2, it was incubated with 5 ml of undiluted rabbit anti-p190 serum plus 5 ml 5% OA/TS overnight at 20 $^{\circ}$ C. After five washes in TS, specifically bound antibodies were eluted by incubation with 2 ml of 0.1 M glycine-Cl, pH 2.6, 0.15 M NaCl (15 min, 20 $^{\circ}$ C). The eluted material was neutralized with 2 ml 1 M Tris-Cl pH 8.0 and 16 ml 5% OA/TS plus 0.01% NaN<sub>3</sub> were added. This solution was used to probe Western blots (lanes 3, 5). Total KI proteins were prepared from parasite pellets ( $\sim 2 \times 10^9$  mixed stage parasites) after saponin lysis of erythrocytes<sup>1</sup>. The pellets were boiled in 500  $\mu$ l of Laemmli sample buffer and after spinning on a microfuge (5 min) 80  $\mu$ l of the supernatant were run on a 7.5% SDS-polyacrylamide gel.

tein(s). Such proteins can be minor contaminants in the p190 preparation used to raise the polyclonal rabbit antiserum.

The hybrid protein of a true p190 clone should be able to bind specific anti-p190 immunoglobulin molecules, permitting them to be affinity purified from the original anti-p190 probe. By contrast, the hybrid proteins of false clones would select immunoglobulin molecules against the contaminating protein.

Hybrid protein from a candidate p190 clone ( $\lambda$ p190-1) was bound to nitrocellulose and used to affinity-purify antibodies from the original probe (Fig. 3). The selected antibodies, when eluted and used to probe Western blots of total parasite proteins, prove to bind to p190 and its processed products (160,000, 110,000, 92,000, 80,000 and 71,000  $M_r$ ). Moreover, the selection is quite specific. When antibodies against another parasite protein (lactate dehydrogenase,  $M_r$  35,000) were added to the anti-p190 probe, these were not selected (compare lanes 4 and



**Fig. 4** Parasite sequences hybridizing to the  $\lambda$ p190-1 cDNA insert. Panel A, Southern blots of *Eco*RI cleaved genomic DNA from different isolates of *P. falciparum*. Lane a, isolate KI (Thailand)<sup>10</sup>; lane b, isolate MAD20 (Papua, New Guinea)<sup>3</sup>; lane c, Tak9 clone 96 (Thailand)<sup>21</sup>. Markers are *Hind*III fragments of  $\lambda$ cI857 (kb). Panel B, Northern blot of total poly(A)<sup>+</sup> RNA from isolate KI. Markers are *E. coli* 16S and 23S RNA and *P. falciparum* '18S' and '28S' RNA<sup>20</sup> (kb).

**Methods:** For Southern blots 6  $\mu$ g of genomic DNA were cleaved with 10 U of *Eco*RI (overnight, 37 $^{\circ}$ C) and electrophoresed on a 0.7% agarose gel. For Northern blots 3  $\mu$ g of poly(A)<sup>+</sup> RNA were run on a 0.8% agarose gel in 2.2 M formaldehyde<sup>20</sup>. Both DNA and RNA were transferred to nitrocellulose in 1 M NH<sub>4</sub>Ac, 0.02 M NaOH (1 h). The probe was prepared by digesting  $\lambda$ p190-1 DNA with *Kpn*I and *Sst*I to yield a 2.4 kb fragment which includes the 300 bp cDNA insert (see Fig. 2A). This was separated from other fragments on a preparative 0.6% agarose gel and after purification, nick-translated with [ $\alpha^{32}$ P]dCTP to a specific activity of  $1.7 \times 10^7$  c.p.m.  $\mu$ g<sup>-1</sup>. After hybridization, filters were washed to a stringency of  $0.1 \times$ SSC at 37 $^{\circ}$ C.

5), nor were antibodies already present in the probe against 42,000, 38,000 and 33,000  $M_r$  contaminating proteins (compare lanes 2 and 3).

Antibody select is a rapid and convenient way to confirm or identify any foreign gene sequences expressed in bacteria. It provides an alternative to both hybrid selection of mRNA<sup>18</sup>, which requires a large amount of mRNA from the parent organism, and immunization of mice with the fusion protein, followed by immuno-identification of the parent protein in extracts<sup>13,19</sup>. This latter approach is slow and we find that fusion proteins do not always elicit a strong immune response. Antibody select has the added advantage that the antibody probe used in the initial identification of the clones provides the immunoglobulins for the confirmation step. It also provides a way to purify from complex sera antibodies against defined regions of a single antigen molecule.

The clones and their hybrid protein are stable during growth in *E. coli*. Their inserts, which can all be excised with *Eco*RI, hybridize strongly to a total cDNA probe (made from blood-stage parasite poly(A)<sup>+</sup> RNA) and give an identical fragment of approximately 300 bp (data not shown). We have therefore studied one clone, representing a small fraction of the protein coding sequence in detail. We can detect the p190 message in total poly(A)<sup>+</sup> RNA extracted from mixed blood stages of the parasite. In Northern blots of denaturing gels<sup>20</sup> it has an apparent length of 6.7 kilobases (kb) (Fig. 4b), which agrees with the estimate that a coding sequence of at least 5 kb would be required for a 190,000  $M_r$  protein.

The gene can be detected in total genomic DNA. When cut with *Eco*RI, it reveals a single major band which hybridizes strongly to the probe (Fig. 4a). Interestingly, when we compare DNA from three different isolates, we find a small but significant difference between KI (4.5 kb) and two others, MAD20 and the

```

      10      20      30      40      50
GAATTCAGTTTAAATTTATTAGAGCAAAATTAATGATGTATGTCTAATGATTAT
GluPheGlnPheAsnLeuLeuArgAlaLysLeuAsnAspValCysAlaAsnAspTyr

      60      70      80      90     100     110
TGTCAAATACCTTTCAATCTTAAATTCGTGCAAAATGAATTAGACGTACTTAAAAAAGT
CysGlnIleProPheAsnLeuLysIleArgAlaAsnGluLeuAspValLeuLysLysLeu

      120     130     140     150     160     170
GTGTTCCGATATAGAAAACCATTAGACAATATTAAAGATAATGTAGGAAAAATGGAGATT
ValPheGlyTyrArgLysProLeuAspAsnIleLysAspAsnValGlyLysMetGluIle

      180     190     200     210     220     230
ACATTAAAAAATAAAAAACCATAGAAAATATAAATTAATTAATTTGAAGAAAGTG
ThrLeuLysLysIleLysLysProIleGluAsnIleAsn***

      240     250     260     270     280     290
TAAGAAAACAATTTGATTAAATTAAGAAATGCCAACTAAAAAATAAAAAAATAAAAA
AAAAAGGAATTC

```

**Fig. 5** Nucleotide sequence of  $\lambda$ p190-1 cDNA. Sequence determination was carried out by the chain termination method<sup>32</sup> after subcloning *EcoRI* fragments of  $\lambda$ p190-1 cDNA into M13mp9 (ref. 33) in both orientations. The putative polyadenylation signal is underlined. The reading frame is shown in phase with  $\beta$ -galactosidase. The two *EcoRI* linkers are indicated by a line above the sequence.

genetically pure clone Tak9/96 (4.7 kb). K1 and Tak9/96 are from Thailand<sup>10,21</sup>, whilst MAD20 is from Papua, New Guinea<sup>3</sup>. This result may be particularly important as MAD20 and Tak9/96 differ from K1 with respect to the antigenic properties of p190<sup>2,3</sup>.

We have estimated the frequency of the cloned p190 sequence in the *P. falciparum* DNA from the intensity of the 4.5 kb band in Southern blots of total genomic digests. Standard copy-number analysis<sup>22,23</sup> using the  $\lambda$ p190-1 clone suggests that the number of copies of the p190 sequence per parasite genome is between one and five (data not shown). The part of the gene in p190-1 does not contain tandem repeats (see below and Fig. 5) as has recently been reported in coding sequences for *P. falciparum* S-antigens<sup>13</sup> and *P. knowlesi* circumsporozoite protein<sup>12</sup>.

Sequencing studies (Fig. 5) in this laboratory on the p190-1 insert reveals a single open reading frame encoding 70 amino acids in phase with  $\beta$ -galactosidase, terminating with a UGA codon followed downstream by two other stop codons. The clone has a run of 26 A residues at its 3' terminus. In addition, the sequence ATAAA starts 24 bases upstream from the first terminal adenine, the expected position for a polyadenylation signal. This function has recently been attributed to the above sequence in several different systems<sup>24-26</sup>. We therefore suggest that the cloned sequence encodes the 3'-terminus of the p190 mRNA and the C-terminus of the polypeptide.

Our conclusion affects the interpretation of the mechanism by which p190 is processed from a primary product of 190,000  $M_r$  to apparently smaller products. Examination of the parasite proteins reacting to the antibodies selected by the p190 clone reveals that in addition to p190, large product bands are also found (Fig. 3, lanes 3, 5). This strongly suggests that epitopes in the C-terminus are not removed by processing. The simplest interpretation of our result is that processing removes the N-terminus of the p190 polypeptide.

We have been unable to detect in the polypeptide encoded in p190-1 epitopes defined by monoclonal antibodies, including 2.2 and 7.3. This may simply reflect the fact that p190-1 contains less than a tenth of the total coding sequence. An alternative explanation is that the C-terminus of p190 cannot elicit an immune response because it is sequestered inside the parasite, as the monoclonal antibodies were obtained after immunization Hwith a whole parasite preparation<sup>2</sup>.

The present report describes a cloned gene for a *P. falciparum* protein known to be both strongly immunogenic in man and a candidate for a subunit vaccine against malaria. We now have

clones with larger p190 sequences which will test whether, Hlike the native protein (and its homologue in *P. yoelii*<sup>3</sup>), p190 synthesized in bacteria can elicit a protective response against blood stages of the parasite.

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## Human antisera detect a *Plasmodium falciparum* genomic clone encoding a nonapeptide repeat

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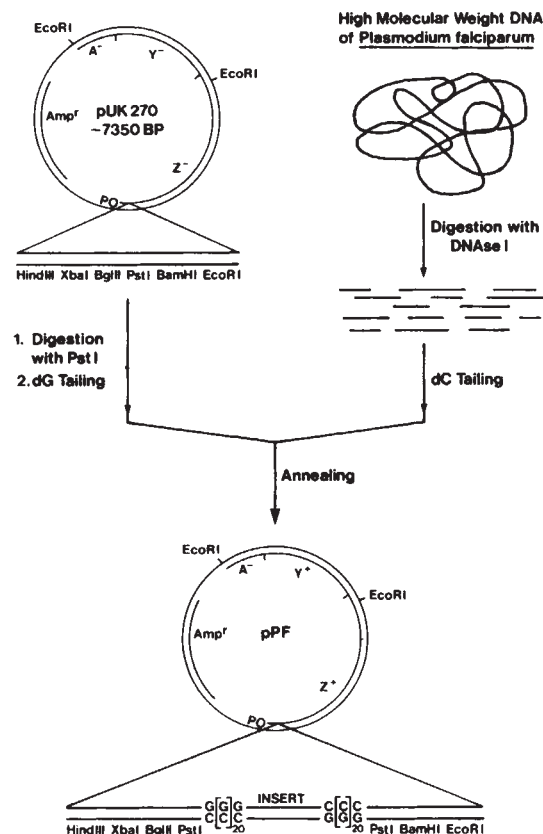
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*Plasmodium falciparum* causes malaria infections in its human host. Its wide distribution in tropical countries is a major world health problem. Before a vaccine can be produced, the identification and characterization of parasite antigens is necessary. This can be achieved by the cloning and subsequent analysis of genes coding for parasite antigens<sup>1-4</sup>. Recently established cDNA banks allow the expression of cDNA derived from the simian parasite *Plasmodium knowlesi*<sup>5</sup> and *P. falciparum*<sup>6,7</sup> in *Escherichia coli*. Recombinants encoding parasite antigens have been identified by immunodetection in both banks. Two of them contain repetitive units of 11 (ref. 7) or 12 (ref. 5) amino acids. We describe here the construction of an expression bank made directly from randomly generated fragments of *P. falciparum* genomic DNA. We detect several clones which react strongly with human African immune sera. One clone expresses an antigenic determinant composed of occasionally degenerated repeats of a peptide nonamer.



**Fig. 1** Cloning of genomic DNA from *P. falciparum* in the expression vector pUK270. The vector pUK270 carries the *lacZ* and *lacY* genes with six unique restriction sites and a frameshift mutation in the promoter proximal part of the *lacZ* gene. Introduction of DNA inserts into the *Pst*I site after dG/dC tailing may overcome the frameshift mutation. Such plasmids will confer a Lac<sup>+</sup> phenotype on *E. coli* hosts which carry a deletion in the *lacZ* gene<sup>8</sup>. Such Z<sup>+</sup> clones can be isolated either as lactose-positive colonies on lactose minimal agar or as blue colonies on rich plates containing isopropyl-1-thio- $\beta$ -D-galactoside as inducer and 5-bromo-4-chloro-3-indolyl- $\beta$ -D-galactoside as indicator. The vector carrying no insert gives a pale blue colour to *E. coli* strains carrying a *lac* deletion on lactose indicator plates but does not allow growth on lactose plates. The lactose-positive recombinants express the inserted DNA as part of the N-terminus of  $\beta$ -galactosidase. Such chimaeric antigen-producing clones can be easily identified if antibodies directed against the expressed antigen are available<sup>8</sup>. Here we have modified the immunoenzymatic detection method described previously<sup>8</sup>. We have used nitrocellulose filters placed on lactose agar for colony replication and growth. Colonies grown on filters were lysed by dipping the filters for 1 min in chloroform at room temperature. Chloroform was removed from the filter by exposure to air for 5 min at 37 °C. Polyvinyl chloride sheets (80 mm diameter) were coated with antibodies and saturated with serum albumin<sup>10</sup>. We used IgG raised in rabbits immunized with Triton X-100 soluble *P. falciparum* asexual blood-stage antigens. The antibody-coated sheets were placed on the lysed colonies. After incubation for 1 h at 4 °C, the sheets were taken off and soaked in wash solution containing 0.5% Triton X-100 for 20 min. The adhering cell debris was removed by squirting with a 20-ml syringe containing wash solution over the sheets. To remove the last traces of cell debris the sheets were placed once more in fresh wash solution. The immunocomplexes were identified as described previously<sup>8</sup>. We used Kemp's method<sup>6</sup>, which needs less antibody, to screen the Lac<sup>+</sup> colonies with a pool of four human African sera with high IFA titres directed against *P. falciparum* (gift of Professor Mannweiler). The mixture of antisera was cleared of antibodies directed against *E. coli* with sonicated extracts of *E. coli*. Immunocomplexes were detected with iodinated Protein A from *S. aureus*<sup>6</sup>. The lysed colonies were incubated with the treated human antisera after a  $\times 25$  dilution.



**Fig. 2** *a*, DNA sequence of the insert of clone pPF11-1; *b*, the derived protein sequence.

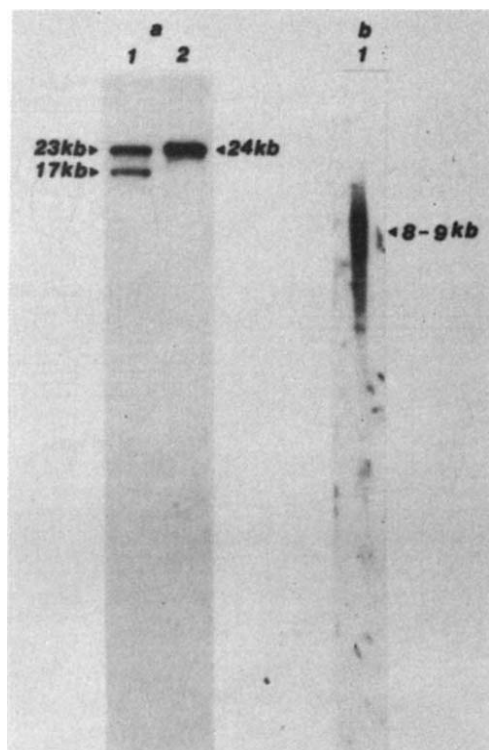
| <i>a</i> |     | 5'... C <sub>n</sub> GGA GAG GTT GTG CCT |
|----------|-----|------------------------------------------|
| Repeat   | 1.  | GAA GAA GTT GTT GAA GAA GTT GTG CCT      |
|          | 2.  | ... T.A ... .. .A .A                     |
|          | 3.  | .T. ... C.A .A ... ..A ...               |
|          | 4.  | .T. ... C.A .A ... ..A .A                |
|          | 5.  | .G ... C.A ... ..G ... A.A ...           |
|          | 6.  | ... A. .G ... .. A.A ...                 |
|          | 7.  | ... .C ... ..A .A ...                    |
|          | 8.  | .G ... A.A ... ..G A.A ...               |
|          | 9.  | .T.A A. ... ..A ...                      |
|          | 10. | .T. ... C.A T.A ... ..A .A .A            |
|          | 11. | .T. ... .A T.A ... .T ... A.A ...        |
|          | 12. | ... .. .A .A .A                          |
|          | 13. | ... A. A. ... ..A .A ...                 |
|          | 14. | ... A. A. ... .G .CG A.A .A              |
|          | 15. | .T. ... A. ... ..G ... A.A .A            |
|          | 16. | ... .T A. ... ..A .A .A                  |
|          | 17. | ... A. ... .T ... A.A .A                 |
|          | 18. | ... .. .A .A ...                         |
|          | 19. | ... .. .A .A .A                          |
|          | 20. | ... T.A .A ... ..A .A .A                 |
|          | 21. | ... C.A ... ..G .G A.A ...               |
|          | 22. | ... CC. ... .G ... A.A                   |
|          | 23. | GAA GAA CTC C <sub>n</sub> ...3'         |

| <i>b</i> |     | --- Glu Val Val Pro                 |
|----------|-----|-------------------------------------|
| Repeat   | 1.  | Glu Glu Val Val Glu Glu Val Val Pro |
|          | 2.  | ... Leu ... ..                      |
|          | 3.  | Val ... Leu ... ..                  |
|          | 4.  | Val ... Leu ... ..                  |
|          | 5.  | ... Leu ... .. Ile ...              |
|          | 6.  | ... Ile ... .. Ile ...              |
|          | 7.  | ... ..                              |
|          | 8.  | ... Ile ... .. Ile ...              |
|          | 9.  | ... Leu Ile ... ..                  |
|          | 10. | Val ... Leu Leu ... .. Ile ...      |
|          | 11. | Val ... Leu ... Asp ... Ile ...     |
|          | 12. | ... .. Ile ...                      |
|          | 13. | ... Ile Ile ... .. Ile ...          |
|          | 14. | ... Ile Ile ... .. Ala Ile ...      |
|          | 15. | Val ... Ile ... .. Ile ...          |
|          | 16. | Asp Ile ... .. Ile ...              |
|          | 17. | ... Ile ... .. Asp ... Ile ...      |
|          | 18. | ... .. Ile ...                      |
|          | 19. | ... ..                              |
|          | 20. | ... Leu ... .. Ile ...              |
|          | 21. | ... Leu ... .. Ile ...              |
|          | 22. | ... Pro ... ..                      |
|          | 23. | Glu Glu Leu ---                     |

We digested high-molecular weight DNA from the Palo Alto Uganda (FUP) strain<sup>4</sup> of *P. falciparum* with DNase I. Fragments of 50–1,000 base pairs (bp) were separated on a polyacrylamide gel and cloned into the 5' end of the *lacZ* gene by dG/dC tailing (see Fig. 1 legend). We used the vector pUK270 that carries in the non-essential 5' end of the *lacZ* gene several unique restriction sites. These sites introduce a frameshift into the *lacZ* gene

which renders the Z, Y and A genes phenotypically Z<sup>-</sup>, Y<sup>-</sup> and A<sup>-</sup>. After cutting the vector with *Pst*I, dG-tailing and annealing with genomic fragments, only recombinants allowing read-through or restart in phase are phenotypically Lac<sup>+</sup>. We identified these positive recombinants and isolated and screened 10,000 Lac<sup>+</sup> clones with antisera (Fig. 1). We screened the clones with rabbit antiserum, obtained after immunization with Triton-

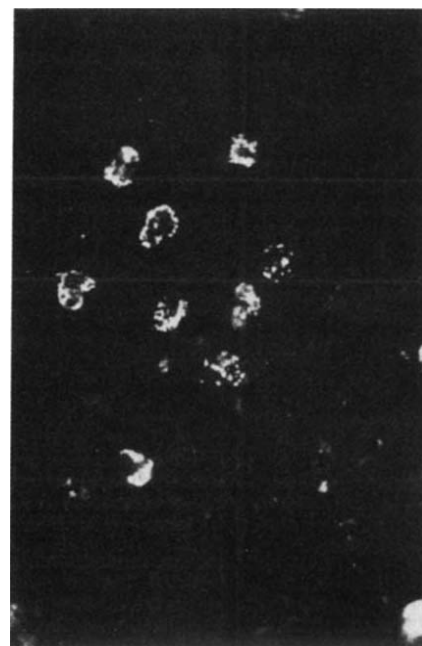


**Fig. 3** *a*, Hybridization of genomic DNA of *P. falciparum* (FUP strain) with the 620-bp insert of clone pPF11-1. The genomic DNA (2 µg DNA per track) was cut with *EcoRI* (track 1) and *HindIII* (track 2). The DNA was fractionated on a 0.8% agarose gel, and transferred from the gel to nitrocellulose paper<sup>13</sup>, hybridized with <sup>32</sup>P-labelled pPF11-1 probe, and autoradiographed. *b*, Hybridization of polyadenylated mRNA isolated from *P. falciparum* (blood stage) with the 620-bp insert of clone pPF11-1. 1.5 µg of polyadenylated mRNA was fractionated on a 1% agarose-formaldehyde gel, transferred to nitrocellulose paper, hybridized, washed and autoradiographed<sup>15</sup>.

extracted proteins from *P. falciparum* asexual blood stages, using an immunoenzymatic technique<sup>8-10</sup>. Colonies producing antigen are visualized directly; we identified 36 positive clones with the rabbit antibodies.

We then screened 10,000 Lac<sup>+</sup> recombinants with a mixture of human sera from adult Africans living in areas endemic for malaria. Here, we visualized immunocomplexes with iodinated Protein A from *Staphylococcus aureus*<sup>6</sup>. Several positive clones were identified, most of them identical to those previously detected with the rabbit antisera. We present here the analysis of one antigen-producing clone, pPF11-1, which reacts strongly with both the rabbit and the human antisera in both tests. It produces a β-galactosidase fusion protein of about 140,000 molecular weight (MW) which is found in cytoplasmic extracts as a stable soluble protein and accounts for approximately 10% of the total protein. This clone contains 620 bp of *P. falciparum* genomic DNA. An insert of this length is consistent with the increase in size of the β-galactosidase fusion protein.

We next determined the complete sequence of the insert by the method of Maxam and Gilbert<sup>11</sup>, and we found a 27-bp repeat running throughout the entire fragment. DNA sequence analysis reveals that only one reading frame contains no stop codons (Fig. 2). This frame codes for the ideal sequence Glu-Glu-Val-Val-Glu-Glu-Val-Val-Pro, but shows little similarity with the 12-amino acid repeat reported for the CS protein of *P. knowlesi*<sup>5</sup> or the 11-amino acid repeat found in *P. falciparum* S-antigen<sup>7</sup>. There is some variation in our polypeptide sequence as alanine, isoleucine or leucine can replace valine and aspartic acid or valine can replace glutamic acid. We conclude that the polypeptide probably exists as an α-helix, possibly interrupted by the prolines. The representation of this presumed α-helix



**Fig. 4** Indirect immunofluorescence of *P. falciparum* antigen with anti-pPF11-1 antibodies. Thin smears of cultured parasites were fixed by acetone. The parasites were incubated with a 1/20 dilution of mouse antiserum raised against SDS-denatured 11-1 proteins and then revealed by fluorescein-conjugated anti-mouse IgG rabbit antibodies. Examination by phase-contrast microscopy (not shown) indicates that the immunofluorescence is not associated with the mature intracellular parasite but with the surrounding red blood cell membrane.

according to Schiffer and Edmundson<sup>12</sup> reveals alternating amphipathic nonapeptides separated by proline residues, which suggests a superstructure of intertwined α-helices.

The pattern generated when pPF11-1 was hybridized<sup>13</sup> to FUP genomic DNA (Fig. 3*a*, track 2) demonstrates homology to a single *HindIII* fragment of approximately 24 kilobases (kb). We can identify two *EcoRI* fragments of 23 and 17 kb (Fig. 3*a*, track 1), and single large *BamHI*, *AhaIII*, *NdeI*, *HinfI* and *BglII* fragments. Sequences homologous to pPF11-1 are found in DNA extracted from a Thai isolate, K1 (ref. 14), and clone T9.96 (ref. 15) (data not shown). Our finding that the inside of pPF11-1 contains no *EcoRI* site, whereas two *EcoRI* fragments are observed in both FUP and T9.96<sup>15</sup> DNA, suggests that the repeated sequence spans more than 620 bp. Furthermore, a transcript of blood-stage *P. falciparum* of approximately 8–9 kb is identified<sup>16</sup> using pPF11-1 DNA as a probe (Fig. 3*b*). A mRNA of this apparent size could encode a protein of approximately 250,000 MW.

We raised rabbit and mice antisera against the purified fusion protein. In late trophozoites and schizont-infected red blood cells (RBC), a parasite protein can be detected by indirect immunofluorescence using these sera. The combination of phase-contrast and fluorescence microscopy indicates that the protein is located in patches associated with the membrane of the infected RBC (Fig. 4) and is not detected in ring-infected RBC or in merozoites. Using the same sera we are unable to immunoprecipitate an antigen from boiled culture supernatant in conditions where immune monkey serum immunoprecipitates an S-antigen of 200–220,000 MW. Preliminary data suggest that the 11-1 antigen has a higher molecular weight and is a minor constituent of the parasite protein. Several antigens are associated with the surface of erythrocytes infected with late stage parasites: schizont-infected cell agglutination antigen<sup>17</sup> which undergoes antigenic variation may be related to sequestration on endothelial cells<sup>18</sup>. We are currently investigating whether pPF11-1 is an antigen of this type.

Finally, concerning the difference between the commonly used cDNA expression banks (see refs 5–7) and our genomic



expression bank, in a cDNA bank the most commonly expressed gene products are overrepresented. In searching for cDNA coding for a rare protein, the overrepresented cDNAs of frequent gene products have to be accounted for. In a genomic bank all genes are equally represented, therefore only one bank is needed for one organism. In cDNA there is a bias for clones coding for the C-terminal end of the particular protein; a genomic bank shows no such bias.

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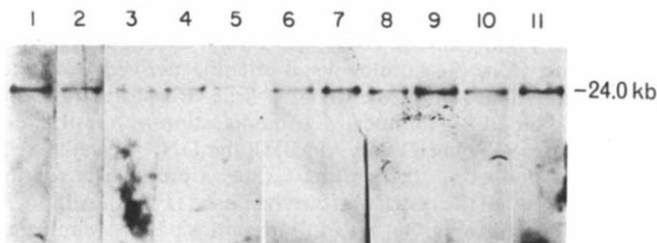
## Rearrangements of T-cell receptor gene *YT35* in human DNA from thymic leukaemia T-cell lines and functional T-cell clones

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An essential property of the immune response is its ability to distinguish between self and non-self and to generate enormous diversity in antibody and T-cell immune responses. Although the genetic and molecular mechanisms responsible for antibody diversity have now largely been elucidated, the structure of the T-cell receptor and the diversification of the receptor repertoire have only recently become amenable to study. One approach has involved immunochemical studies of the protein precipitated by monoclonal antibodies which react specifically with the immunizing T-cell clones<sup>1–3</sup>. Another approach has been to clone and sequence a human<sup>4</sup> or a murine<sup>5</sup> T-cell specific message that may specify part of the T-cell receptor. We present here results of Southern blot analysis of non-T, immature T, and mature T-cell genomic DNA, and provide evidence that rearrangements of the *YT35* sequences do occur in the DNA of thymic leukaemia T cells. This suggests that *YT35* codes for at least part of the T-cell receptor and that rearrangements occur at this early stage of thymic ontogeny. Furthermore, DNA rearrangements are present in lymphocytes with phenotypic and functional characteristics of helper, killer, or suppressor T cells. We conclude that the three subpopulations of T cells operate via receptor molecules encoded by the same gene family.



**Fig. 1** Southern gel analysis of the T-cell receptor gene *YT35* in non-T-cell DNA. DNA was extracted from 11 independent samples of non-T cells and digested with 3 units of *Bam*HI per  $\mu$ g DNA overnight. DNA was then electrophoresed through 0.8% agarose gel as previously described<sup>19</sup>. DNA was transferred to nitrocellulose filters and hybridized to <sup>32</sup>P-dCMP-labelled nick-translated C35, a *Hpa*I (nucleotide 430) to *Eco*RV (nucleotide 1,087) subclone of *YT35* (ref. 4) by the method of Southern<sup>6</sup>. For detailed procedure, see ref. 19. Lane 1, DNA from granulocytes of a donor; lane 2, DNA from fibroblasts of the donor; lane 3, DNA from B-cell line RPMI 3638; lane 4, DNA from myeloid leukaemia cell line HL-60; lanes 5–11, DNAs from fibroblast cultures from seven different donors.

In order to determine the germ-line organization of the gene(s) corresponding to the putative T-cell receptor cDNA *YT35* and to estimate the frequency of DNA polymorphism among individuals, we have used Southern blot analysis<sup>6</sup> on 23 samples of genomic DNA extracted from fibroblasts (15 samples), B-cell lines (five lines), a bladder tumour cell line MGHU-1 and myeloid cell lines (two lines). The DNA samples were digested with the restriction enzyme *Bam*HI and probed with C35, a constant-region subclone of *YT35*. One 24-kilobase (kb) restriction fragment band was observed in each of the non-T cells examined (see Fig. 1). We also obtained similar results showing unchanging patterns and intensities of restriction fragment bands in non-T-cell DNA using four other restriction enzymes, *Bst*EII, *Hind*III, *Eco*RI and *Sph*I. These data establish that the non-T-cell DNAs from a variety of sources show no rearrangement of sequences corresponding to the putative T-cell receptor cDNA clone *YT35*. It also appears that DNA polymorphism between individuals is rare for sequences homologous to the *YT35* constant region.

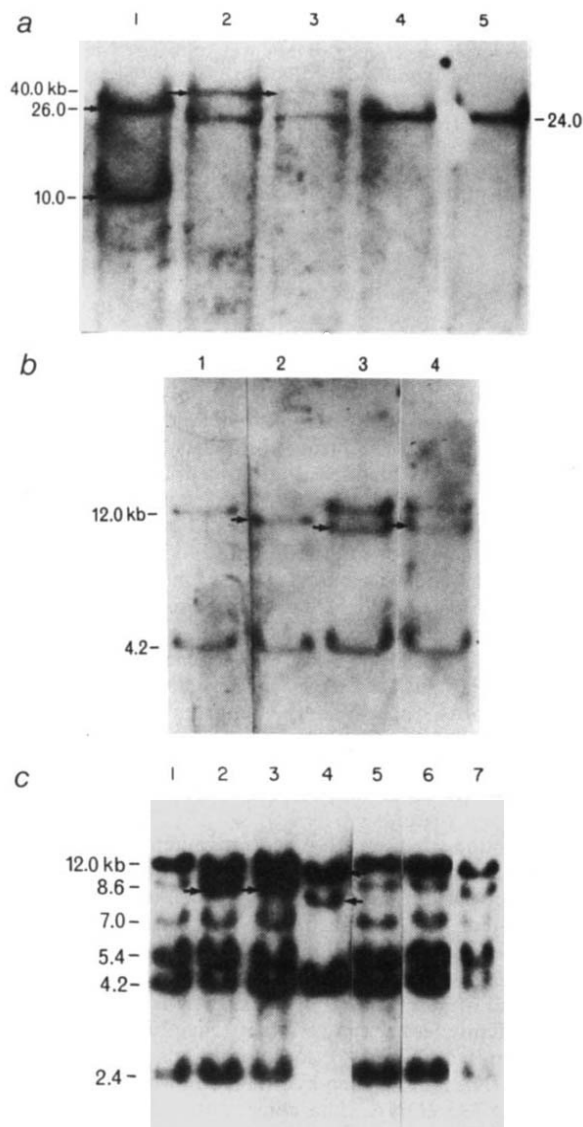
To determine whether DNA rearrangements occur during or after intrathymic differentiation, we next investigated the rearrangement patterns of several thymic leukaemia lines. DNA was extracted from leukaemic T-cell lines and treated as described above. Our results show that DNA of non-T cells (Fig. 2a, lanes 4 and 5) contain the same restriction fragment band typical of germ-line DNA as summarized in Fig. 1. DNA from the thymic leukaemia cell lines (Fig. 2a, lanes 1, 2 and 3; Fig. 2b, lanes 2, 3 and 4) contains bands not found in the germ-line DNA lanes. When hybridization was carried out using the entire *YT35* cDNA, data consistent with DNA rearrangement in T-cell DNA were also observed, but the number of bands increased to 6 or 7 (Fig. 2c, lanes 2–4). In addition to the germ-line bands presented by the C35 probe, hybridization with the entire cDNA *YT35* probe reveals four new bands corresponding to the homologous variable gene segments.

Regulatory (helper and suppressor) and effector (killer) T-lymphocytes probably cooperate via complex interactions to establish a successful immune response, but the existence and relative role of the T-cell receptor in each of these subpopulations is unknown. In order to ascertain the presence of the T-cell receptor in functionally distinct T-cell subpopulations, we analysed four mature T-cell clones with helper, killer or suppressor function for possible gene rearrangements of sequences corresponding to the putative T-cell receptor clone *YT35*. All these T-cell clones were derived from the same individual and were generated by *in vitro* alloactivation against stimulating cells from a single donor<sup>7,8</sup>.

The biological activities of these T-cell clones are summarized in Table 1. Total genomic DNA was extracted from these T-cell clones and from the autologous B-cell line derived from the same individual by Epstein-Barr virus transformation of peripheral blood B-lymphocytes. After digestion with restriction enzyme (*Eco*RI, *Bam*HI and *Hind*III), the DNA was analysed by the method of Southern using C35 as a probe. The results are similar for all the restriction enzymes used (Fig. 3). Although the B-cell bands (Fig. 3a, b, lane 1) retain a pattern similar to the germ-line DNA (Fig. 1), all four T-cell-clone DNA samples exhibit rearrangements of DNA homologous to YT35 (Fig. 3a, b,

lanes 2-5). Our results indicate that T-cell clones with helper, killer or suppressor activity exhibit gene rearrangement of sequences corresponding to the putative T-cell receptor gene YT35. The use of twin clones derived from the same individual excludes the possibility that the rearrangement is a reflection of an individual characteristic trait.

The results presented here are consistent with the somatic rearrangement of genomic T-cell DNA sequences corresponding to the putative human T-cell receptor cDNA clone YT35. The unchanging or germ-line restriction fragment band patterns found in the numerous non-T-cell DNA samples (Fig. 1) indicate

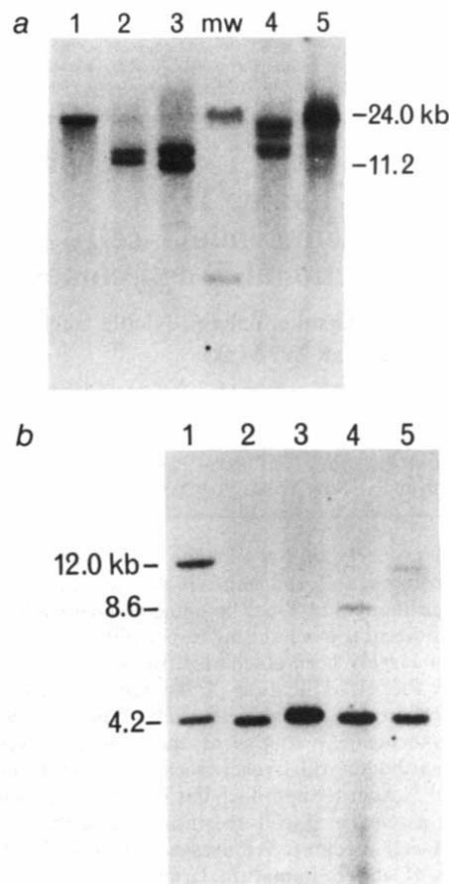


**Fig. 2** Southern gel analysis of T-cell receptor gene YT35 in DNA from thymic leukaemia lymphoblastic cell lines. DNA from thymic leukaemia cell lines MOLT-3, Jurkat and CEM, and control DNA from four non-T sources were analysed by Southern gel analysis (described in Fig. 1) using either the C35 constant-region probe (a, b) or the entire YT35 cDNA (ref. 4) (c) as a probe. Arrows indicate rearranged bands. a, DNA digested with *Bam*HI and hybridized with C35 constant region as a probe. Lane 1, CEM; lane 2, Jurkat; lane 3, MOLT-3; lane 4, RPMI 3638; and lane 5, HL-60. b, DNA digested with *Eco*RI and hybridized with C35 constant region as a probe. Lane 1, fibroblast from a donor; lane 2, CEM; lane 3, Jurkat; lane 4, MOLT-3. c, DNA digested with *Eco*RI and hybridized with the entire YT35 (ref. 4) as a probe. Lane 1, fibroblast from a donor; lane 2, MOLT-3; lane 3, Jurkat; lane 4, CEM; lane 5, RPMI 3638; lane 6, HL-60; lane 7, bladder tumour cell line MGJU-1.

**Table 1** Characterization of functional T-cell clones

| Clone | Phenotype | T-cell functions |      |               |
|-------|-----------|------------------|------|---------------|
|       |           | Blastogenic      | CML  | PFC formation |
| 19    | OKT4      | DR1(r.086)       | None | Helper        |
| 22    | OKT4      | DR1(r.083)       | None | Helper        |
| 209   | OKT4      | DR1(r.083)       | None | Suppressor    |
| 26    | OKT8      | None             | B35  | No effect     |
| 207   | OKT4      | DR1(0.83)        | DR1  | Helper        |

T-cell phenotype was determined by cytofluorimetric testing of reactivity with murine monoclonal antibody reacting with human T-cell surface antigens<sup>16</sup> (Ortho Diagnostic). Blastogenic response was determined in a 92-h <sup>3</sup>H-TdR incorporation assay. Cell-mediated lymphocytolysis (CML) was ascertained by <sup>51</sup>Cr-release assay<sup>17</sup>. Plaque-forming cells (PFC) induced by T-cell clones were tested as described<sup>18</sup>.



**Fig. 3** Southern gel analysis of T-cell receptor gene YT35 in DNA from five mature functional T-cell clones. DNAs and an autologous Epstein-Barr virus-transformed B-cell clone from the same individual were digested with *Eco*RI or *Bam*HI and examined by Southern gel analysis using C35 constant region as a probe (described in Fig. 1). a, *Bam*HI; b, *Eco*RI. Lane 1, LBCL (B-cell line); lane 2, clone 209 (suppressor); lane 3, clone 22 (helper); lane 4, clone 207 (helper and killer); lane 5, clone 19 (helper).



that DNA polymorphism is infrequent in genomic sequences homologous to *YT35*. The *YT35*-encoding protein thus resembles the  $\kappa$  light chains of mammalian immunoglobulin, where DNA polymorphism is not common<sup>9</sup>. It is possible that polymorphism gives rise to the invariant restriction fragment band patterns of thymic leukaemic T-cell DNA (Fig. 2a-c), because we did not have the autologous non-T-cell DNA samples for comparison. To rule out this possibility, we observed DNA rearrangements in several mature T-cell clones where the autologous B cells from the same individual retain the germ-line patterns. These results (Fig. 3) suggest that DNA rearrangement in leukaemic T cells is not due to DNA polymorphisms among individuals. The increased complexity of the restriction band pattern observed on probing with the entire cDNA *YT35* (variable and constant regions) (Fig. 2c) implies the existence of multiple variable segments in the genome which is consistent with data analysing the germ-line genomic DNA (G. Siu *et al.*, unpublished observations). Thus, it appears that the generation of T-cell receptor diversity is accomplished by a rearrangement mechanism similar to that found in immunoglobulin production<sup>10,11</sup>. Immunoprecipitation of T-cell membrane glycoprotein by use of clonotypic monoclonal antibodies suggests the existence of two distinct chains in the T-cell receptor molecule<sup>1-3</sup>. *YT35* appears to code for one of these polypeptide chains, and further diversity can be expected to emerge through interactions with the other chain.

The non-germ-line patterns observed in monoclonal thymic leukaemia cell lines clearly indicate that rearrangement of the putative T-cell receptor gene *YT35* occurs during intrathymic differentiation in ontogenesis. Recent data demonstrate that the T-cell receptor for alloantigens is probably the most primitive one<sup>12</sup>, and is also associated with the receptor for well-defined soluble antigens<sup>13,14</sup>. Our finding that helper, suppressor and cytotoxic T-cell clones exhibit rearrangement of DNA fragments corresponding to the *YT35* cDNA clone demonstrates that this gene codes for a receptor molecule which is present in all T-cell subpopulations. Furthermore, the level of mRNA expression of the cDNA *YT35* clone has been found to decrease following post-thymic differentiation of helper, suppressor and cytotoxic T cells<sup>15</sup>. Our data, combined with these findings, show that the *YT35* clone corresponds to a T-cell receptor gene and that rearrangement of this gene is involved in the diversification of the T-cell receptor repertoire.

Receptor gene rearrangement occurs in early ontogenetic stages of intrathymic differentiation, when T cells acquire the capacity to recognize both self and allogeneic MHC antigens. Rearrangement can also be found in helper, killer and suppressor T cells, suggesting that the three classes of cells operate via receptor molecules encoded by the same gene family. We hope that the isolation of the T-cell receptor gene from human and mouse will help in dissecting these complex interactions between lymphocytic and non-lymphocytic cells necessary for successful humoral and cytolytic immune responses.

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## Identification of a diversity segment of human T-cell receptor $\beta$ -chain, and comparison with the analogous murine element

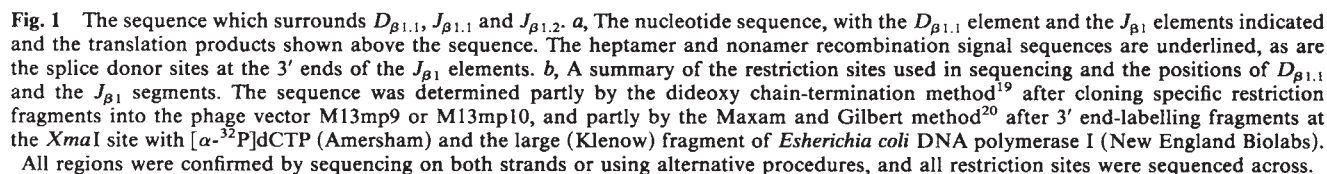
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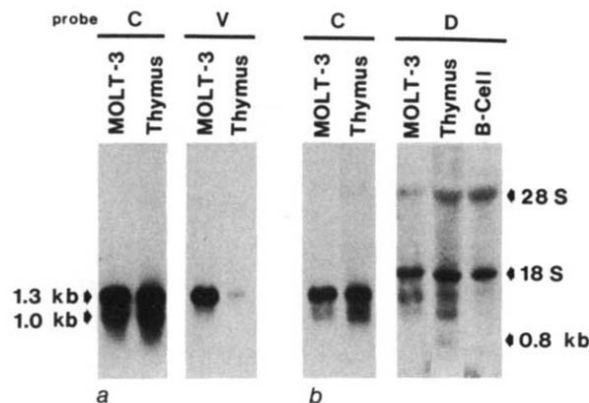
The humoral immune system antigen-binding proteins (immunoglobulins) are disulphide-linked heterodimers of light and heavy chains. The gene for the variable region which determines antigen specificity is assembled when one member from each of the dispersed clusters of variable (*V*) gene segments, diversity (*D*) elements (for the heavy chains only) and joining (*J*) segments rearrange and fuse during B-cell development (reviewed in ref. 1). Short recognition sequences adjacent to these elements appear to be involved in the recombination process. The cellular immune system antigen recognition proteins are receptors on the surface of T cells, which are composed of disulphide-linked  $\alpha$ -chains and  $\beta$ -chains, each of which has a variable and constant region<sup>2-4</sup>. Recently, cDNA clones of the  $\beta$ -chain<sup>5</sup> mRNA have been isolated<sup>6,7</sup>; the genomic arrangement is very similar to immunoglobulin genes with multiple *V<sub>β</sub>* genes<sup>8</sup>, and two clusters of *J<sub>β</sub>* segments, each of which is upstream from a constant-region gene segment<sup>9</sup>. The *V<sub>β</sub>* and *J<sub>β</sub>* segments have adjacent recombinational recognition sequences like the immunoglobulin elements. However, ~10 nucleotides of the cDNA clones between the *V<sub>β</sub>* and *J<sub>β</sub>* regions were not present in the corresponding genomic elements and may have been due to intervening *D<sub>β</sub>* segments<sup>8,10</sup>. Here we describe a diversity element (*D<sub>β1.1</sub>*) in a region of high human-mouse homology about 650 bases 5' to the first *J<sub>β</sub>* cluster. Two transcripts which include sequences upstream of *D<sub>β1.1</sub>* are found in the human thymus. This region may have some other function besides providing the  $\beta$ -chain with a diversity segment.

We have extended our previous sequence<sup>8</sup> of *J<sub>β1</sub>*, the first of the two human *J<sub>β</sub>* clusters, from 750 bases 5' to *J<sub>β1.1</sub>* to 380 bases 3' to *J<sub>β1.2</sub>* (Fig. 1a). This cluster is about 4 kilobases (kb) 5' to *C<sub>β1</sub>*. We identified what we believe to be a diversity element, *D<sub>β1.1</sub>*, 650 bases upstream from *J<sub>β1.1</sub>*. Its position with respect to the *J<sub>β</sub>* cluster is similar to that of the murine immunoglobulin heavy-chain *D* element, *D<sub>Q52</sub>* (ref. 11) and the equivalent human element, *D<sub>HQ52</sub>* (ref. 12). However, there is no obvious homology between *D<sub>β1.1</sub>* and the human or murine *D<sub>Q52</sub>* element, other than the recombination signal sequences which flank the elements and the stretch of guanosine residues in the 3' half of the protein coding region (comparisons not shown). *D<sub>β1.1</sub>* has the typical recombination recognition heptamer and nonamer sequences<sup>13,14</sup> separated by 12 bases on the 5' side and 23 bases on the 3' side (Fig. 1a). This structure was expected because the germ-line *V<sub>β</sub>* gene has a 23-base spacer between the two signal sequences and the *J* segments have a 12-base spacer (Fig. 1 and refs 8-10) and indicates that the  $\beta$ -chain follows the same 12/23 spacer rule for recombination that governs the



Sequences 5' to the presumptive coding region of  $D_{\beta 1.1}$  are present in the 1.0-kb transcript of immature thymocytes. Such transcripts are also found in the mouse<sup>17</sup>. An open reading frame extends upstream in the human and murine  $D_{\beta}$  segments, so it may be that these short transcripts encode a protein with





**Fig. 2** Analysis of thymus and MOLT-3 RNA for transcripts containing  $V_{\beta}$  sequences,  $C_{\beta}$  sequences or 5'  $D_{\beta 1.1}$  flanking sequences. RNA was extracted from the cells, electrophoresed and transferred to nitrocellulose membranes as previously described<sup>6</sup>. *a*, Hybridization of a YT35 constant- (*C*) or variable-specific (*V*) fragment. *b*, Hybridization with the YT35 constant-region probe (*C*).

**Methods:** *a*, The *C* or *V* fragment was gel-purified, nick-translated and hybridized to the nitrocellulose in hybridization solution (50% deionized formamide,  $5\times\text{SSC}$  ( $1\times\text{SSC}$  is 150 mM NaCl, 15 mM Na citrate, pH 7.0), 50 mM sodium phosphate, 250  $\mu\text{g ml}^{-1}$  salmon sperm DNA,  $1\times\text{Denhardt's}$  solution) and  $1\times 10^6$  c.p.m. per ml of probe at 40 °C for 16 h. The final wash was in  $0.1\times\text{SSC}$ , 0.1% SDS at 50 °C. *b*, The method for hybridization was as for *a*. After 80 h of autoradiography, the filter was washed three times in  $0.1\times\text{SSC}$ , 0.1% SDS at 90 °C, then exposed to film for 60 h to ensure complete removal of the probe. The 1.3-kb band was barely visible. The filter was then hybridized to the 30-base oligonucleotide probe  $D_{\beta A30}$  (*D*; complementary to positions 93–64 in Fig. 1*a*, including 12 bases of  $D_{\beta 1.1}$  and 18 bases of the adjacent 5' flanking region) which had been 5' end-labelled with [ $\gamma$ - $^{32}\text{P}$ ]ATP (Amersham, specific activity 3,000 Ci mmol $^{-1}$ ) and  $T_4$  polynucleotide kinase (Boehringer Mannheim). Hybridization was performed in hybridization buffer with  $1\times 10^6$  c.p.m. per ml at 37 °C for 16 h, then at 21 °C for 24 h. The filters were washed twice with  $5\times\text{SSC}$  at 21 °C and exposed for 10 days. The control lane of B-cell RNA shows that  $D_{\beta A30}$  strongly hybridizes to 28S and 18S RNA, and that the 1.3-kb and smaller messages are specific to T cells.

unknown properties and functions. A prediction arising from this hypothesis is that there should be no homology 3' of the  $D_{\beta}$  region, which would be deleted during recombination with a  $J_{\beta}$  segment. This is indeed seen when murine  $D_{\beta 2.1}$  is compared with the other two  $D_{\beta}$  elements, but not when the human and murine  $D_{\beta 1.1}$  elements are compared (Table 1). Perhaps the 0.8-kb transcript observed in the thymus includes regions 3' to  $D_{\beta 1.1}$ , or possibly there is some transcript being produced on the opposite strand, where there are long open reading frames in the human and murine  $D_{\beta 1.1}$  regions, but not in the murine  $D_{\beta 2.1}$ .

The presence of a prominent and discrete transcript of 1.0 kb in immature but not mature T cells from both man (Y.Y. *et al.*, in preparation) and mouse<sup>17</sup> suggests that this message has a function during T-cell development. The frameshifts upstream from the  $D_{\beta}$  elements suggest that any proteins resulting from distinct incomplete  $D$ - $J$  rearrangements would be quite different, so might be involved in the generation of, or selection for, receptor specificity. Unfortunately, knowledge of the molecular biology of the processes of T-cell ontogeny is scarce, so it is difficult to suggest a function for this RNA species or the protein it may encode. Further characterization of the 1.0-kb transcript may allude to some of these processes, and might indicate a reason for the strong homology around  $D_{\beta 1.1}$  between species.

**Table 1** Homology between human and murine  $D_{\beta}$  elements and their flanking sequences

|                        | Upstream of 5' nonamer | Between nonamer recombination signals | Downstream of 3' nonamer |
|------------------------|------------------------|---------------------------------------|--------------------------|
| Human $D_{\beta 1.1}$  | 78%                    | 90%                                   | 68%                      |
| Murine $D_{\beta 1.1}$ |                        |                                       |                          |
| Human $D_{\beta 1.1}$  | 60%                    | 75%                                   | Random                   |
| Murine $D_{\beta 2.1}$ |                        |                                       |                          |
| Murine $D_{\beta 1.1}$ | 54%                    | 79%                                   | Random                   |
| Murine $D_{\beta 2.1}$ |                        |                                       |                          |

|                                                                           |                      |
|---------------------------------------------------------------------------|----------------------|
| 114: CAGCTTATCTGGTGGTTTCTCCAGCCCTCAAGGGTAG, ACCTATGGG, AGGGTCTTTTGTATATAA | Murine D $\beta$ 1.1 |
| 1: CAGCTCTCTGGTGGTTCTCTCCAGGC, TCTGGGGCGG, ACCATGGG, AGGGGTGTTTGTATATAA   | Human D $\beta$ 1.1  |
| 86: CACCTGTCTCCTCCCTGCCAGGC, TCTGGGGTAGGCACCTGTGGGAAGAACTTTTGTATAC        | Murine D $\beta$ 2.1 |
| 114: CAGCTTATCTGGTGGTTTCTCCAGCCCTCAAGGGTAG, ACCTATGGG, AGGGTCTTTTGTATATAA | Murine D $\beta$ 1.1 |
| 182: GCTGTAACTTGTGGGACA, GGGGGCACGGTATTCAATCTATGGGAAGCCTTACAAAACTT        | Murine D $\beta$ 1.1 |
| 68: GCTGTAACTTGTGGGACA, GGGGGCACAAATGATTCACTCTACGGGAAGCCTTACAAAACTT       | Human D $\beta$ 1.1  |
| 155: GATGTAACTTGTGGGACA, GGGGGCACAAATGATTCACTCTACGGGAAGCCTTACAAAACTT      | Murine D $\beta$ 2.1 |
| 182: GCTGTAACTTGTGGGACA, GGGGGCACGGTATTCAATCTATGGGAAGCCTTACAAAACTT        | Murine D $\beta$ 1.1 |
| 250: CTGTCTGTCCCAAGGCCACAGTCTCTACTCTCTCTGGTGGCAAGCCTAGATGTGTTAGATCCAG     | Murine D $\beta$ 1.1 |
| 136: CTGGGGTCCCAACTCCAGAGTCTCTCTCTCTGGTGGCAAGCCTTAATGCAATTTGGTTCAG        | Human D $\beta$ 1.1  |
| 225: ACCCAAAAAACCAACTCTCAGCCCAAAAAATGCAATTAACATGTGAGGAGAGCTATATGAGTGA     | Murine D $\beta$ 2.1 |
| 250: CTGTCTGTCCCAAGGCCACAGTCTCTACTCTCTCTGGTGGCAAGCCTAGATGTGTTAGATCCAG     | Murine D $\beta$ 1.1 |
| 320: AATGCTTTCAGCTCACCTCTGCAG                                             | Murine D $\beta$ 1.1 |
| 206: AATGCTTTCAGCTCACCTCTGCAG                                             | Human D $\beta$ 1.1  |
| 295: CTCACAGGCTTATACATCTAT                                                | Murine D $\beta$ 2.1 |
| 320: AATGCTTTCAGCTCACCTCTGCAG                                             | Murine D $\beta$ 1.1 |

**Fig. 3** Comparison of the human  $D_{\beta 1.1}$  and the murine  $D_{\beta 1.1}$  and  $D_{\beta 2.1}$  regions. The murine sequences are from Siu *et al.*<sup>17</sup>. Solid circles indicate identity between adjacent sequences, and dots show where gaps have been introduced to improve the alignment. The relevant regions are marked.

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# Isolation of UV-resistant revertants from a xeroderma pigmentosum complementation group A cell line

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Xeroderma pigmentosum (XP) is an autosomal recessive disease. Cells cultured from XP patients are hypersensitive to the lethal effects of UV light<sup>1-6</sup>. Most XP cells are defective in an early stage in DNA repair of UV light-induced damage. The nature of the genetic defect of the XP syndrome has not been defined. To address this problem, we attempted to isolate UV-resistant cells from a cell line derived from an XP complementation group A (XPA) patient. By using a selection scheme capable of detecting one UV-resistant cell in a population of  $10^8$  cells, several UV-resistant clones were isolated at frequencies between  $1 \times 10^{-7}$  and  $2 \times 10^{-8}$ . Here we describe the isolation and initial characterization of these phenotypic revertants.

We used the cell line designated M1 which has been characterized with respect to its histocompatibility locus antigen (HLA) type, karyotype, isoenzyme profile, restriction map of the integrated simian virus 40 (SV40) genome, and its ability to function as a recipient in transfection experiments<sup>7</sup>. Multiple exposures to UV light were used to select for UV-resistant clones. The frequency of occurrence of UV-resistant clones in different experiments (listed in Table 1) was in the range  $1 \times 10^{-7}$  to  $2 \times 10^{-8}$ , regardless of whether the cells had been exposed to DNA under transfection conditions, subjected to mock transfection, or subjected to selection without previous treatment. The observation that the frequency of appearance of UV-resistant colonies was the same regardless of the previous treatment of the cells indicates that the frequency of reversion to a UV-resistant phenotype is substantially higher than the frequency of DNA-mediated gene transfer of the wild-type XPA allele in the conditions used. We have been unable to repeat the results of Takano *et al.*<sup>8</sup> using their cell line as recipient, the same source of donor DNA, and the same selection conditions. In control experiments we found that the transfection frequency of a single-copy *gpt* gene integrated into HeLa cell DNA was  $2 \times 10^{-6}$  per  $20 \mu\text{g}$  of high-molecular weight DNA, indicating that transfer of single-copy genes can be detected using the methods outlined here.

Several tests were done to determine whether the UV-resistant cell lines were derived from the original M1 UV-sensitive parent. The morphology, doubling time and cloning efficiency of the UV-resistant cell lines were indistinguishable from those of the M1 culture. In all cases tested, the restriction map of the integrated SV40 genome was the same as that of the M1 culture (Fig. 1). The isoenzyme profile, HLA type and the karyotype are also identical to those of the parental M1 cell line (Table 2). We conclude that the UV-resistant colonies derived in these experiments are phenotypic revertants of the M1 XPA culture.

The cytotoxic effect of UV-irradiation of several of the UV-resistant colonies was compared with that of normal and SV40-transformed human fibroblasts. Figure 2 shows that although  $D_0$  varied somewhat from clone to clone, they all were within the normal range. ( $D_0$  is the dose required to reduce survival by  $1/e$  on the exponential portion of the survival curve.)

The ability of several cell lines to support the replication of UV-irradiated herpes simplex virus was examined<sup>9</sup>. The plating efficiency of UV-irradiated herpes simplex virus on the resistant cell lines is comparable with that on normal cell lines and distinctly different from that of the M1 cells (Fig. 3a). Similar results were obtained using UV-irradiated SV40 DNA in transfection assays<sup>10</sup>. One revertant cell clone, XP-RH10, was tested

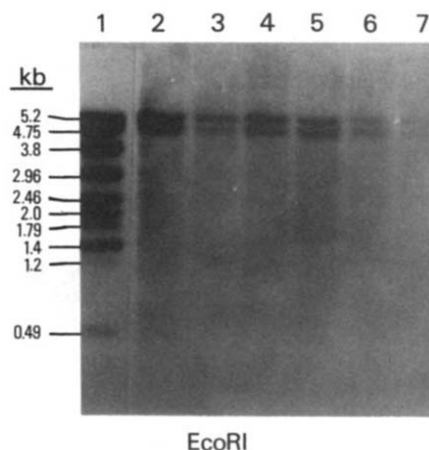


Fig. 1 Characterization of integrated SV40 DNA. DNA was isolated from the parental M1 cell line and from the revertants indicated below. The DNA was digested to completion with *EcoRI* and the sizes of the *EcoRI* DNA fragments determined by Southern blot analysis using SV40 DNA as probe, as described previously<sup>7</sup>. Lane 1, size markers (SV40 DNA digested with *EcoRI*, *HhaI*, *PstI*, *PvuII*, *TaqI* and *HpaII*, as described elsewhere<sup>7</sup>). Lane 2, parental XP12RO-M1 clone; 3, XP-RE1; 4, XP-RH3; 5, XP-RH10; 6, XP-RG3; 7, XP-RG1.

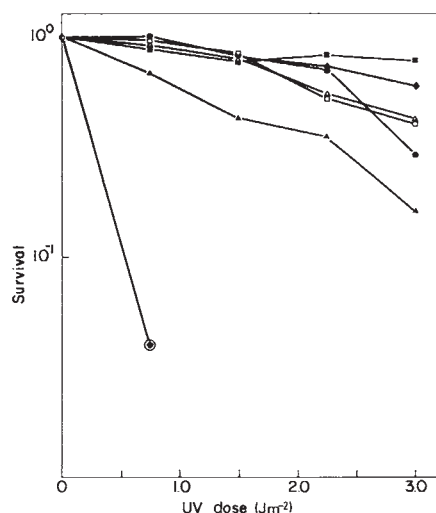


Fig. 2 Dose-response of M1 and revertant cell lines. ●, XP12RO-M1; ▲, XP-RG1; ●, XP-RE1; □, XP-RH10; △, XP-RH12; ■, 1522, normal human fibroblasts; ◆, GM637, SV40-transformed fibroblasts. 1,000 cells were seeded per 60-mm dish and irradiated after 24 h under a layer of 1 ml Hank's buffer. After removal of the Hank's buffer, growth medium containing 15% serum was added. The medium was changed after 1 week and the plates were stained after 2 weeks. Colonies were counted and the relative survival determined as:

$$\frac{\text{number of colonies on UV-irradiated plates}}{\text{number of colonies on unirradiated plates}}$$

The relative survival values were found to be the same between experiments that were repeated at least three times for several of the clones. For the determination of  $D_0$  values, plates were irradiated with higher doses of UV.

and found to resemble the normal cells rather than the M1 cell line. We conclude that the UV-resistant cell lines tested have recovered the ability to carry out host cell reactivation.

The ability of the cells to remove cyclobutane pyrimidine dimers from DNA can be measured by determining the number of UV-specific endonuclease-sensitive sites in DNA at several times post-irradiation. For this study, cells were irradiated with  $2.5 \text{ J m}^{-2}$ ; DNA was extracted immediately and 24 h after

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**Table 1** Frequency of occurrence of UV-resistant M1 clones

| Experiment | Treatment               | No. of cells used | No. of revertants | Designation of clones | Frequency               |
|------------|-------------------------|-------------------|-------------------|-----------------------|-------------------------|
| a          | None                    | $1 \times 10^7$   | 0                 |                       | $<1 \times 10^{-7}$ *   |
| b          | None                    | $5.3 \times 10^7$ | 11                | XP-RB1-XP-RB11        | $2 \times 10^{-7}$      |
| c          | None                    | $3 \times 10^8$   | 11                | XP-RC1-XP-RC11        | $3.6 \times 10^{-8}$    |
| c          | CaPO <sub>4</sub> +DMSO | $2.6 \times 10^7$ | 4                 | XP-RC12-XP-RC16       | $1.5 \times 10^{-7}$    |
| e          | CaPO <sub>4</sub> +DMSO | $1 \times 10^7$   | 2                 | XP-RE1, XP-RE2        | $2 \times 10^{-7}$      |
| f          | CaPO <sub>4</sub> +DMSO | $1 \times 10^7$   | 2                 | XP-RF1, XP-RF2        | $2 \times 10^{-7}$      |
| g          | XP DNA                  | $1 \times 10^7$   | 2                 | XP-RG1, XP-RG2        | ND†                     |
| k          | XP DNA                  | $5 \times 10^6$   | 0                 |                       | $<2 \times 10^{-7}$ *   |
| h          |                         | $1 \times 10^7$   | 9                 | XP-RH1-XP-RH9         | $9 \times 10^{-7}$ ‡    |
| g          | Normal human DNA        | $1 \times 10^7$   | 7                 | XP-RG3-XP-RG10        | ND†                     |
| k          |                         | $5 \times 10^6$   | 0                 |                       | $<2 \times 10^{-7}$ *   |
| g          | Mouse DNA               | $6.5 \times 10^6$ | 3                 | XP-RG11-XP-RG14       | ND†‡                    |
| h          | Mouse DNA               | $3 \times 10^6$   | 2                 | XP-RH10, XP-RH11      | $6 \times 10^{-7}$ ‡    |
| k          | Mouse DNA               | $3.5 \times 10^6$ | 0                 |                       | $<8.5 \times 10^{-7}$ * |

To select for UV-resistant clones,  $2 \times 10^7$  M1 cells were seeded at a density of  $4 \times 10^6$  cells in large plates (245 cm<sup>2</sup>). Selection was begun with a UV dose of  $1 \text{ J m}^{-2}$  24 h after seeding. The selective pressure was maintained by exposure to  $0.5 \text{ J m}^{-2}$  every other day for 2 weeks. At the end of this time cells were exposed to  $1 \text{ J m}^{-2}$  and allowed to grow for 1 week. Surviving colonies were picked and tested for UV resistance. Only colonies having a  $D_0 > 2.2$  were scored as resistant in this assay. Reconstruction experiments using M1 and SV40-transformed normal human cells demonstrate that this selection method can detect 1 in  $10^8$  UV-resistant cells. The frequency represents the number of UV-resistant colonies per initial number of cells plated. Between the start of transfection experiments and plating out for UV selection, the cells doubled two or three times. For different treatments, the same letter (a, b, c, e, f, g, h or k) indicates that the experiments were done at the same time, for example in expt c, one part of a pool of cells was subjected to UV selection alone and another part to calcium phosphate + dimethyl sulphoxide (DMSO) plus UV selection. In a typical transfection experiment,  $5 \times 10^5$  cells were plated on 60-mm plates, then treated either with  $20 \mu\text{g}$  DNA per plate or with CaPO<sub>4</sub> precipitate alone, or left untreated. DNA was left on the plates for 24 h. Cells were then treated with 10% DMSO for 10–20 min. The medium was changed every day for 5 days and cells were split to low density on day 6 ( $3 \times 10^6$ – $1 \times 10^7$  cells per dish, 245 mm<sup>2</sup>). The next day UV selection was initiated as described above. In experiments g and k, cells were split the day after DMSO treatment and UV selection was initiated on day 3 after addition of DNA.

\* No UV-resistant clones were obtained for the number of cells plated, therefore the frequency is less than the indicated number.

† ND, not determined. In this experiment the frequency could not be calculated, as the colonies obtained after UV selection were pooled and reseeded before a second round of UV selection.

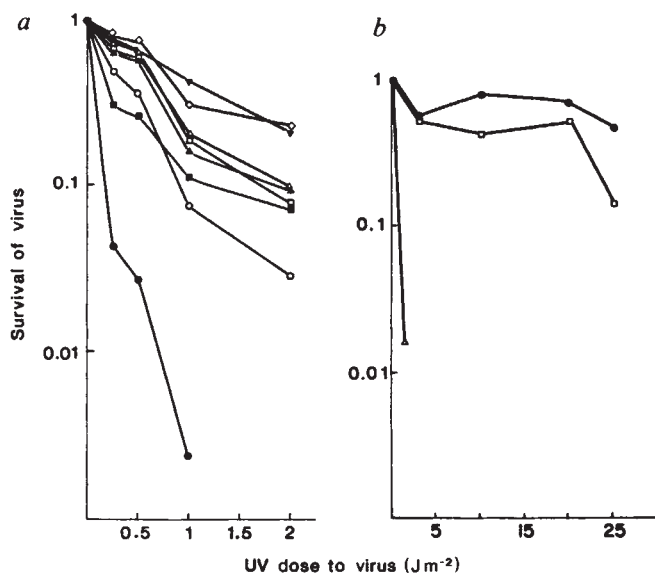
‡ The clones were analysed for donor DNA by Southern blotting. In the case of clones transfected with mouse DNA, DNA from the revertant clones was analysed for repetitive mouse DNA as described by Grusella *et al.*<sup>15</sup>. No repetitive murine sequences were found in the UV-resistant cells. XP-RH1-XP-RH9 clones were transfected with normal human DNA ligated to pSV2gpt DNA, which codes for resistance to mycophenolic acid (MPA). The clones contained neither pBR322 sequences nor additional SV40 sequences, and were not resistant to MPA (data not shown). These results indicate that the UV-resistant cells contained no transfected DNA.

**Table 2** Karyotype and isoenzyme analysis of the parental XP clone and the UV-resistant (UV<sup>r</sup>) revertants XP-RE1 and XP-RG1

|                              | Parental SV40 XP12RO-M1 clone | UV <sup>r</sup> clones XP-RE1 and XP-RG1 |
|------------------------------|-------------------------------|------------------------------------------|
| <b>Cytogenetics</b>          |                               |                                          |
| Sex                          | Male                          | Male                                     |
| Near-diploid count           | 44%                           | 30%                                      |
| Near-tetraploid count        | 56%                           | 62%                                      |
| Highly polyploid             | 0                             | 2%                                       |
| Marker A (3q <sup>+</sup> )  | +                             | +                                        |
| Marker B (14q <sup>+</sup> ) | +                             | +                                        |
| <b>Isozymes</b>              |                               |                                          |
| LDH                          | Human                         | Human                                    |
| G6PD                         | B                             | B                                        |
| PGM1                         | 1–2                           | 1–2                                      |
| PGM3                         | 1                             | 1                                        |
| ESD                          | 1                             | 1                                        |
| Me-2                         | 1                             | 1                                        |
| Ak-1                         | 1                             | 1                                        |
| GLO-1                        | 1–2                           | 1–2                                      |

The methods used for karyotype, isoenzyme and HLA type analysis have been described previously<sup>7</sup>. LDH, lactate dehydrogenase; G6PD, glucose 6-phosphate dehydrogenase; PGM1 and PGM3, phosphoglucomutase-1 and -3; ESD, esterase D; Me-2, malic enzyme, mitochondrial; Ak-1, adenylate kinase-1; GLO-1, glyoxylase 1.

exposure. The DNA was treated with the UV-specific endonuclease isolated from *Micrococcus luteus*<sup>11</sup>. The size of the DNA samples after such treatment was analysed on alkaline agarose gels and the extent of removal of cyclobutane pyrimidine dimers from the DNA was calculated using the procedures described by Sutherland<sup>12</sup>. Removal of dimers from the DNA of the revertants was not distinguishable from that of wild type cells by this test.



**Fig. 3** Host cell reactivation of herpes virus (a) and SV40 (b). a: ●, XP12RO-M1; ○, XP-RE2; ■, GM637; □, SV80; ▲, XP-RE1; △, XP-RH10; ▼, XP-RH1; ◇, XP-RG3. For host cell reactivation of UV-irradiated herpesvirus,  $7.5 \times 10^5$  cells were seeded on 35 mm plates. The following day, medium was removed and 0.2 ml of herpesvirus (UV-irradiated with the indicated doses) was added and the cells were incubated with the virus for 30 min at 37 °C. Then, 3 ml of medium containing 0.7% agar were added; 4–5 days later, when plaques were visible under the microscope, a fresh layer of medium containing agar and 0.015% neutral red was added. Plaques were counted either 24 or 48 h later. b: △, XP12RO-M1 cells; ●, CV-1 cells; □, XP-RH10. Host cell reactivation of UV-irradiated SV40 was done as described previously<sup>10</sup>.

The ability of several of the isolates to undergo unscheduled DNA synthesis, both as measured by incorporation of  $^3\text{H}$ -thymidine after irradiation of stationary cells and by counting grains overlying the nuclei, was determined as described by Royer-Pokora *et al.*<sup>7</sup> These experiments showed that all the revertants tested (XP-RH10, XP-RG1, XP-RH2 and XP-RE1) were capable of unscheduled DNA synthesis.

Here we have demonstrated that phenotypic revertants can be isolated from the M1 (XP12RO) culture of XPA cells, in agreement with preliminary reports by others<sup>13,14</sup>. Phenotypic reversion may arise by several mechanisms including back mutation of the primary genetic defect, second-site compensatory mutations within the gene, suppression either by nonsense or missense suppressors, increased activity of a secondary pathway, or the overexpression of a defective gene product. The frequency of UV-resistant colonies observed in these experiments, between  $1 \times 10^{-7}$  and  $2 \times 10^{-8}$ , is comparable with reversion frequencies observed for other mutations. The observation that the revertants

resemble the normal cells in all properties tested favours the possibility of a simple back mutation within the gene itself. Nonetheless, these experiments do not exclude other possible mechanisms of reversion. However, if phenotypic reversion has occurred by expression of a different repair mechanism, then this mechanism must resemble that which is defective in XPA cells with respect to certain biochemical properties, for example, excision of cyclobutane pyrimidine dimers.

The revertant cell lines isolated here should provide a convenient source of biological material for a future analysis of the defects of the XPA locus. These cell lines should be isogenic with respect to all properties except for those encoded by the XPA locus itself.

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## Genetic effects of injection of Rous sarcoma virus DNA into polar plasm of early *Drosophila melanogaster* embryos

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Retroviral proviruses and the transposable elements of eukaryotic genomes are structurally similar<sup>1–3</sup>. The biological significance of eukaryotic transposable elements has not been examined extensively but it is known that, like prokaryotic transposons, these elements can induce mutations in adjacent genes and cause their transposition<sup>4,5</sup>. It is of interest to determine whether retroviral proviruses have the same mutagenic<sup>6</sup> and gene transposing ability as transposable elements, particularly because the retrovirus genome is assumed to have originated from transposable elements of lower eukaryotes<sup>1</sup>. The transfer of DNA sequences into animal zygotes or embryos by microinjection is a promising experimental approach for elucidating their functions<sup>7–11</sup>: when foreign DNAs were introduced into a mouse germ line, mutations were induced<sup>9–11</sup> and at least in some mice, the mutation was caused by the insertion of a retroviral sequence<sup>9,10</sup>. We have introduced Rous sarcoma virus (RSV) DNA into a germ line of *Drosophila melanogaster*, and describe here the resultant genetic effects.

Retroviruses are evolutionarily remote from *Drosophila*. Nevertheless, when RSV was added to their food, *Drosophila* larvae and flies were found to carry such anomalies as necroses, tumours and chromosomal rearrangements. The progeny of these flies had recessive lethal mutations but no stable visible mutant lines were isolated, nor was the presence of the virus or its DNA detected in subsequent generations<sup>12</sup>.

By using a microinjection technique, RSV or its cloned DNA fragment was injected into the polar plasm of *Drosophila* embryos at the stage when the nuclei of future germ cells migrate in that area (70–80 min post-oviposition, p.o.). We thus isolated

a set of mutant lines in some of which the virus-specific sequence was detected by Southern blot hybridization. As this stage of embryogenesis takes only 10–15 min, we used a multi-dose injector<sup>13</sup> basically similar to that used by Spradling and Rubin<sup>7</sup>, which allows ~1,000 microinjections per hour, so that several hundreds of synchronously laid eggs can be treated in a single experiment.

In the first series of experiments, we microinjected suspensions of 50–100 intact RSV infectious particles per embryo and among the F<sub>2</sub> progeny we observed some individuals with an altered eye structure, which gave rise to the *eye-deformed* mutant line<sup>14,15</sup>. Hybridization in solution revealed the presence of the virus-specific sequence in the DNA of the mutant flies (~1 copy of the viral genome per *Drosophila* haploid genome). It was unclear, however, whether the appearance of this mutation was accidental, or a result of the microinjection. In subsequent experiments (described below), we obtained a set of new mutant lines (Tables 1 and 2).

In some experiments we injected a fragment of RSV circular cDNA cloned in the pBR322 plasmid, pPrC 11 (see ref. 16 and the accompanying paper<sup>17</sup>). The use of this plasmid instead of RSV increased the yield of mutations (probably because in this way we introduced more DNA than can be formed in the embryonic cytoplasm by reverse transcription of the introduced viruses). The results of DNA analyses of several mutant lines are given in the accompanying paper<sup>17</sup>. The mutants analysed were: Dr-RSV<sup>a</sup>, Dr-RSV<sup>b</sup> (see Fig. 1a), Dr-RSV<sup>c</sup> and Dr-PrC<sup>d</sup> (see Fig. 1b and Table 2), the first three of which are allelic mutations that arose independently. The Dr-PrC<sup>d</sup> mutant has a complex phenotype characterized by three types of wing anomaly: (1) dark, bubble-like wings; (2) wings with various outgrowths; and (3) wings spread out. We failed to isolate these in separate lines, as all three anomalies occurred in the progeny of each pair of flies originally carrying only one of the anomalies. It is interesting that the viral sequence is present in an un-integrated form (plasmid) in the DNA of Dr-PrC<sup>d</sup> flies (ref. 17).

It is possible that the mutations observed in our experiments arose from genome rearrangements induced by exogenous DNA. If so, change in the level of recombination in the embryos at the stage of introduction of viral DNA might affect the mutation rate. To test this notion, we exposed the embryos to UV-light. We had found previously that irradiation of *Drosophila* embryos at stage 12 with short-wave (254 nm) UV light at  $0.005 \text{ J cm}^{-2}$



**Table 1** Results of microinjection of RSV or DNA preparations into embryos of *Drosophila melanogaster*

| Material injected                   | No. of embryos injected | No. of surviving flies | Mutation found in F <sub>2</sub> progeny of a F <sub>0</sub> fly |
|-------------------------------------|-------------------------|------------------------|------------------------------------------------------------------|
| RSV*                                | 1,050                   | 100                    | Dr-RSV <sup>a</sup> (+)                                          |
| RSV*                                | 1,123                   | 111                    | Dr-RSV <sup>b</sup> (+)                                          |
|                                     |                         |                        | Dr-RSV <sup>c</sup> (+)                                          |
| pPrC 11*                            | 640                     | 96                     | Dr-PrC <sup>a</sup> (?)                                          |
|                                     |                         |                        | Dr-PrC <sup>b</sup> (-)                                          |
|                                     |                         |                        | Dr-PrC <sup>c</sup> (-)‡                                         |
|                                     |                         |                        | Dr-PrC <sup>d</sup> (+)                                          |
| pPrC 11†                            | 280                     | 30                     | Dr-UV <sup>a</sup> (?)§                                          |
|                                     |                         |                        | Dr-UV <sup>b</sup> (?)                                           |
| pPrC 11 + UV-irradiation†           | 1,050                   | 21                     | Dr-UV <sup>c</sup> (?)§                                          |
|                                     |                         |                        | Dr-UV <sup>d</sup> (?)                                           |
|                                     |                         |                        | Dr-UV <sup>e</sup> (?)                                           |
|                                     |                         |                        | Dr-UV <sup>f</sup> (?)                                           |
|                                     |                         |                        | Dr-UV <sup>g</sup> (?)                                           |
| pPrC 11 + UV-irradiation†           | 980                     | 14                     | Dr-UV <sup>h</sup> (?)§                                          |
|                                     |                         |                        | Dr-UV <sup>i</sup> (?)                                           |
|                                     |                         |                        | Dr-UV <sup>k</sup> (?)                                           |
| DNA from Oregon R (wild type)†      | 400                     | 35                     | —                                                                |
| DNA from Oregon R + UV-irradiation† | 890                     | 40                     | —                                                                |
| DNA from Oregon R (wild type)*      | 790                     | 85                     | —                                                                |
| Rat liver DNA*                      | 700                     | 79                     | —                                                                |
| pBR322*                             | 850                     | 91                     | —                                                                |
| Eagle's medium*                     | 1,350                   | 120                    | —                                                                |
| H <sub>2</sub> O*                   | 280                     | 41                     | —                                                                |

Eggs collected during a 15 min interval were used in each experiment. Either 50–100 infectious particles of RSV in Eagle's medium, or  $2-4 \times 10^5$  copies of the pPrC 11 plasmid containing the RSV DNA insert (see ref. 16 and the accompanying paper<sup>17</sup>) or an adequate amount of other DNA (as indicated), all in 0.2–0.4 nl, were microinjected using a multi-dose injector<sup>13</sup> into the polar plasm of embryos at the 70–80 min p.o. (25 °C) stage. The flies (F<sub>0</sub>) that developed from the embryos were crossed individually with wild-type Oregon R, and the progeny of each pair were intercrossed to obtain the F<sub>2</sub> generation. F<sub>2</sub> flies having an altered phenotype were individually crossed with wild-type Oregon R flies; the F<sub>3</sub> progeny of each pair were intercrossed, then F<sub>4</sub> individuals with anomalies were used to obtain stable mutant lines (lethals and non-heritable anomalies were discarded by the crossing procedure). Independently obtained mutants of similar phenotype were tested for allelism by the standard complementation procedure. Brackets enclose one complementation group. Symbols in parentheses represent the results of analysis of the DNA for the presence of the RSV sequence (see accompanying paper<sup>17</sup> and ref. 18): +, sequence was detected; –, sequence was not detected; ?, line not analysed.

\* Embryos used were wild-type Oregon R.

† Embryos used were mutant stock *wsn*; embryos were totally irradiated with UV (254 nm, 0.005 J cm<sup>-2</sup>) 1 h after the microinjection.

‡ Dr-PrC<sup>c</sup> proved unstable, giving rise to Dr-PrC<sup>e</sup> (see Table 2).

§ Dr-UV<sup>a</sup>, Dr-UV<sup>c</sup> and Dr-UV<sup>h</sup> proved unstable: the mutants obtained from them are being analysed.

increased the yield of recombination in the *wsn* stock by ~80% (S.D.N., unpublished observations). UV-irradiation (0.005 J cm<sup>-2</sup>) of *wsn* embryos that had been injected with pPrC 11 increased several fold the frequency of occurrence of the same mutation that had been induced by the plasmid without exposure to UV (Table 1).

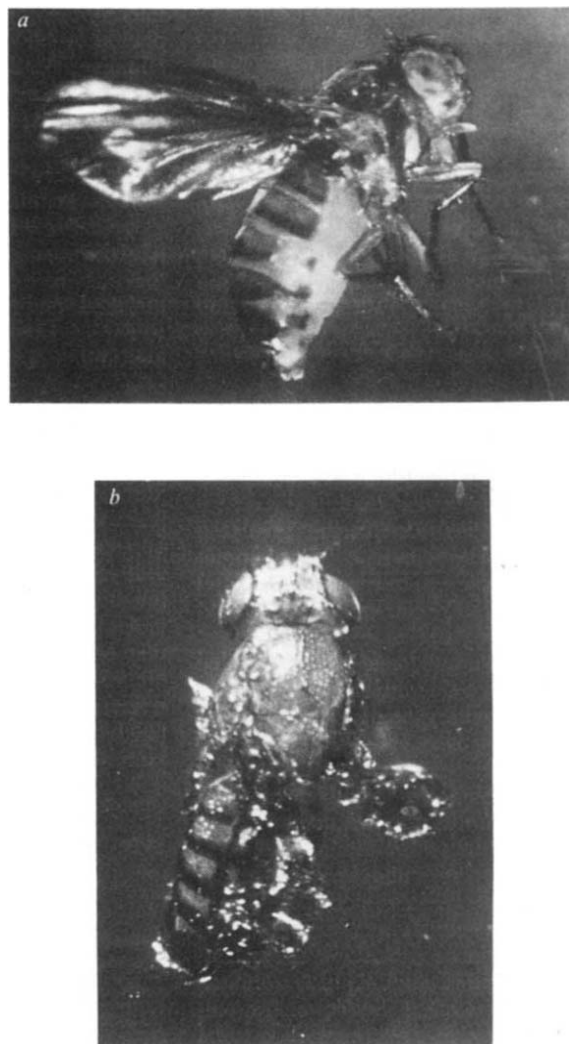
We obtained 18 mutant lines, five of which were unstable (one new mutation appeared spontaneously in each of these). The mutagenesis is clearly selective, and certain loci are preferred; it is unclear, however, whether this depends on the loci themselves or whether it is determined entirely by the mutagen, because the molecular mechanism of the mutations remains obscure. Virus-specific sequences were detected in five of seven mutant lines analysed (including the *eye-deformed* line described previously<sup>13,14</sup>). However, there is no reliable evidence that the mutations actually resulted from the insertion of virus-specific

**Table 2** Characteristics of mutant lines obtained after microinjection of RSV or its cloned DNA into early embryos of *Drosophila melanogaster*

| Mutation              | Phenotype                                                                               | Type of mutation, chromosomal localization*   |
|-----------------------|-----------------------------------------------------------------------------------------|-----------------------------------------------|
| Dr-RSV <sup>a-c</sup> | Tergites diminutive, with uneven edges (see Fig. 1a)                                    | Recessive; chromosome 2; expressed in ♀ only  |
| Dr-PrC <sup>a</sup>   | Wings showing disturbed venation, fusion of M <sub>4+5</sub> and R <sub>1+2</sub> veins | Expressed in ♂ only; decreased viability      |
| Dr-PrC <sup>b</sup>   | Black body pigmentation                                                                 | Recessive; chromosome 3; expressed in ♂ and ♀ |
| Dr-PrC <sup>c</sup>   | Wings showing shiny oval spots                                                          | Recessive; chromosome 2; expressed in ♂ and ♀ |
| Dr-PrC <sup>d</sup>   | Wings showing three phenotypic anomalies (see text and Fig. 1b)                         | Dominant; chromosome 2; expressed in ♂ and ♀  |
| Dr-PrC <sup>e</sup> † | Tergites very small, some lacking                                                       | Dominant; chromosome 2; expressed in ♂ and ♀  |
| Dr-UV <sup>a-k</sup>  | Tergites diminutive with uneven edges, some arranged cross-wise                         | Recessive; chromosome 2; expressed in ♂ and ♀ |

\* For chromosome analysis, the mutants were crossed with Oregon R and *Cy/Pm*; *Ubx/Sb*.

† Dr-PrC<sup>e</sup> was found among 6th-generation flies of the Dr-PrC<sup>c</sup> line (see Table 1).



**Fig. 1** Phenotypic appearance of flies of the mutant lines carrying the viral sequence (see accompanying paper<sup>17</sup>). *a*, Dr-RSV<sup>a</sup>; *b*, Dr-PrC<sup>d</sup> (the variant with outgrowths on the wings; see text).

sequences in the loci concerned, because our attempts to reveal the viral sequence in the polytene chromosomes, by hybridization *in situ*, were unsuccessful. We also considered the possibility that viral DNA derepressed endogenous elements of instability, activating them at their genomic sites or causing their transposition, a notion consistent with our major findings, namely that: (1) UV-irradiation, while increasing the general level of instability (gene recombinations), increases the frequency of plasmid-induced mutagenesis only at the locus which is already mutated without exposure to UV; (2) mutagenesis is highly selective, that is, some loci behave as 'hotspots' in the genome. It is clear, however, that some structural properties of RSV DNA are important in the development of these mutations because neither homologous DNA nor pBR32 showed any mutagenic effect (Table 1).

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## Detection of virus-specific sequences in *Drosophila melanogaster* mutants induced by injection of RSV DNA into early embryos

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The injection of purified Rous sarcoma virus (RSV) (Prague strain) into *Drosophila melanogaster* (Oregon R line) eggs changes the fly phenotype in certain cases, and RSV-specific sequences can be identified in the *Drosophila* genome (ref. 1 and preceding paper<sup>2</sup>). Here we have used Southern blotting<sup>3</sup> to analyse in greater detail the proviral DNA present in several mutant lines of *D. melanogaster* produced by microinjection of intact RSV or plasmid DNA containing the viral insert. In certain populations of flies, RSV provirus was found to be incorporated into cellular DNA, and in one mutant family the unintegrated form of plasmid DNA was identified. Generally, the presence of injected genetic material in fly cells correlated with morphological changes in *Drosophila*.

DNA from mutant and wild-type lines was isolated by phenol extraction<sup>1</sup> and analysed by Southern blotting<sup>3</sup>; the morphological characteristics of the mutant *Drosophila* lines are described in detail in the accompanying paper<sup>2</sup>. Here we present restriction analyses of three of the mutant lines, which are quite different with respect to their viral sequence arrangements.

In the first restriction analysis, DNA was isolated from a mutant *D. melanogaster* population (designated Dr-RSV<sup>a</sup>)

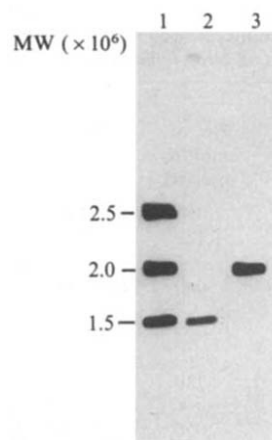


Fig. 1 Presence of RSV-specific sequences in the Dr-RSV<sup>a</sup> genome. DNA was extracted from 1 g of flies (F<sub>8-10</sub> generations) as described previously<sup>1</sup> and 15 µg were digested with *Eco*RI in standard conditions recommended by the supplier. Fragments were separated by electrophoresis on a 1% agarose gel. The DNA was denatured, transferred to nitrocellulose as described by Southern<sup>3</sup> and hybridization was performed in 50% formamide, 4×SSC at 41 °C. The probes ( $1.2 \times 10^8$  c.p.m.  $^{32}$ P µg<sup>-1</sup>) were: lane 1, RSV cDNA, prepared on 35S RSV RNA with calf thymus DNA as a primer<sup>7</sup>; lane 2, nick-translated pPrC-gag DNA containing the  $1.5 \times 10^6$  dalton fragment of *Eco*RI-digested RSV provirus; lane 3, nick-translated pPrC-src DNA containing the 3' part of the RSV genome located between the *Eco*RI and *Hind*III sites<sup>4</sup>.

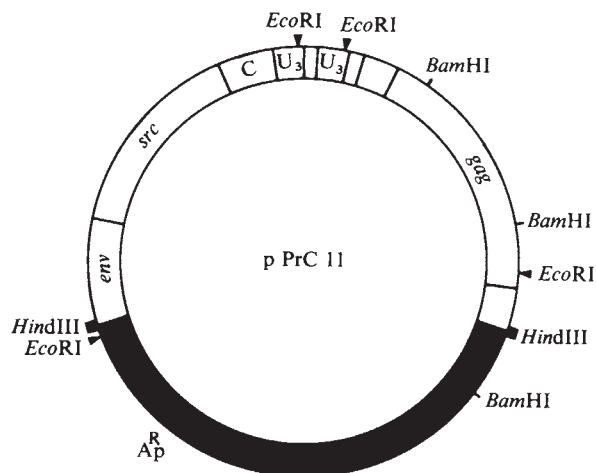
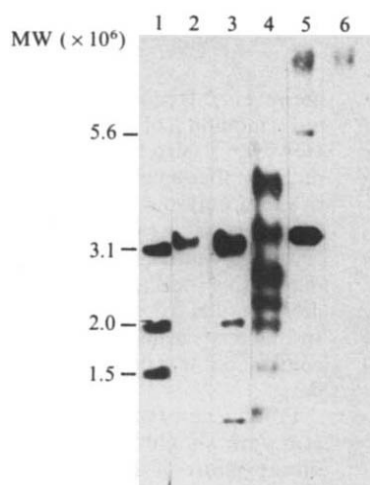


Fig. 2 Structure of pPrC 11 (ref. 4). Circular RSV (Prague strain) DNA was cloned on the *Hind*III site of pBR322. The total molecular weight of pPrC 11 is  $6.9 \times 10^6$  daltons; the viral genome represents  $4 \times 10^6$  daltons. This plasmid contains intact *src* and *gag* genes, two LTRs, the 5' end of *pol* and the 3' part of RSV *env* gene.

induced by microinjection of purified RSV. The DNA was digested with *Eco*RI and, after immobilization on a nitrocellulose filter, either hybridized with total RSV cDNA or cloned fragments of viral genome containing *src* or *gag* sequences<sup>4</sup>.

It is well known that *Eco*RI digestion of the RSV genome (Prague C strain) yields three major so-called internal fragments of molecular weight  $1.5 \times 10^6$ ,  $2 \times 10^6$  and  $2.5 \times 10^6$ . The two smaller fragments contain *gag* and *src* sequences respectively, while the  $2.5 \times 10^6$  dalton fragment is localized in the middle of the genome and contains the *pol* gene and the 5' part of *env*<sup>5</sup>. Figure 1 shows that a total DNA probe detected all three virus-specific fragments in the mutant line Dr-RSV<sup>a</sup> (lane 1); the *gag* probe detected the  $1.5 \times 10^6$  dalton fragment (lane 2); and the *src* probe the  $2.0 \times 10^6$  dalton fragment (lane 3). These



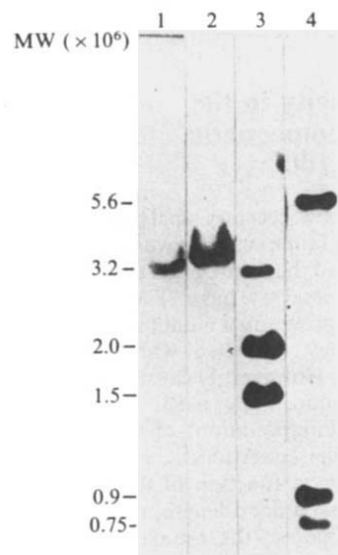


**Fig. 3** The presence of pPrC 11 sequences in *Drosophila* 3A population and the Dr-PrC<sup>d</sup> mutant line (F<sub>8-9</sub> generations). 15 µg of DNA from each fly family were separated on a 1% agarose gel. Lanes 2, 3 and 5, Dr-PrC<sup>d</sup> DNA; lanes 4, 6, *Drosophila* 3A DNA. Lane 1 contains 0.5 µg of pPrC 11 DNA. DNAs in lanes 1–4 were digested with *Eco*RI, DNAs in lanes 5 and 6 received no restriction endonuclease treatment. The probes were: lanes 1, 3–6, nick-translated pPrC 11 DNA; lane 2, RSV cDNA. Lanes 2 and 3 represent the same nitrocellulose filter which was hybridized first with RSV cDNA and, after dehybridization in 60% formamide at 70 °C, rehybridized with nick-translated pPrC 11 DNA.

data show that in this mutant *Drosophila* line, RSV provirus is co-linear with the well known genetic structure of the viral genome<sup>5</sup>. This DNA may persist in an integrated or extrachromosomal form, but our preliminary results with *Bam*HI and *Hind*III restriction lead us to suggest that provirus is associated with chromosomal DNA (data not shown).

The second example of interaction was observed on analysis of the DNA from the mixed progeny of 11 flies obtained after microinjection of bacterial plasmid pPrC 11 (*Drosophila* 3A family). pPrC 11 contains a fragment of circular RSV DNA with two long terminal repeats (LTRs) inserted in the *Hind*III site of pBR322 (Fig. 2). This fly population was not controlled by genetic analysis because we could not obtain a stable line. Compared with the Dr-RSV<sup>a</sup> mutant line, the arrangement of virus-specific sequences in this population is much more complex (Fig. 3, lane 4) and essentially different from the genetic map of plasmid pPrC 11 (Fig. 2, Fig. 3, lane 1). These data show that plasmid DNA containing the viral insert can be integrated in the *Drosophila* genome. As we have been unable to obtain lines from such flies, we have not analysed further the integrated sequences.

Another example of interaction of pPrC 11 with the *Drosophila* genome was observed during analysis of the mutant line, Dr-PrC<sup>d</sup>; this family consists of flies with dominant mutations influencing wing structure and producing three characteristic types (see accompanying paper<sup>2</sup>). Analysis of Dr-PrC<sup>d</sup> DNA showed the presence of deleted plasmid DNA in which RSV sequences were observed. The main hybridization band after *Eco*RI digestion of fly Dr-PrC<sup>d</sup> DNA was equivalent to a size of  $3.2 \times 10^6$  daltons; the intensity of this band on autoradiography was much greater than that of other bands (Fig. 3, lanes 2, 3). On the basis of these results, we suggest that injected DNA may persist in fly cells of this population as an extrachromosomal element, and analysis of Dr-PrC<sup>d</sup> DNA that had not been digested with restriction enzymes supported this suggestion. In this case, we also observed pPrC 11 sequences in the band of  $3.4 \times 10^6$  daltons (Fig. 3, lane 5). In some experiments we found an additional band of  $5.6 \times 10^6$  daltons, which may represent an open circular form of unintegrated DNA (Fig. 3, lane 5).



**Fig. 4** Comparative restriction enzyme analysis of pPrC 11 and pDr 2A DNAs. 0.5 µg of each plasmid was digested with *Eco*RI (lanes 1 and 3) or *Bam*HI (lanes 2 and 4) then, after electrophoresis and blotting, hybridized with RSV cDNA. Lanes 1, 2, pDr 2A DNA; lanes 3, 4, pPrC 11 DNA.

In an analogous experiment, DNA from the 3A family that had not been digested with restriction enzymes showed no indications of persistence in an unintegrated form (Fig. 3, lane 6).

We have tried to 'rescue' the unintegrated form by transformation of *Escherichia coli* HB101 strain with total DNA isolated from the mutant line, Dr-PrC<sup>d</sup> (ref. 6). Ampicillin-resistant *E. coli* were produced which contained a new plasmid designated pDr 2A. The structure of this plasmid is essentially different from that of the original plasmid pPrC 11 (Fig. 4); its detailed structure will be described elsewhere, but it has a molecular weight of  $3.4 \times 10^6$  daltons, and except for LTRs, it does not contain the main part of the RSV sequence. The possibility of insertion of *Drosophila* sequences into this plasmid has been excluded by blot hybridization experiments with DNA from the Oregon R line which gave negative results (data not shown), although we cannot exclude the possibility that a few oligonucleotide sequences from the *Drosophila* genome are scattered within this plasmid.

Our preliminary results suggest that injection of the pDr 2A plasmid into *Drosophila* embryos induced offspring carrying different mutations which produced great variations in phenotype. We conclude, therefore, that microinjection of RSV or its cloned DNA into *D. melanogaster* embryos can lead to different types of proviral DNA interaction with fly cells—persistence of the intact viral genome, integration of a rearranged viral genome and existence of the viral genome in an unintegrated form.

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## 15-Myr periodicity in the frequency of geomagnetic reversals since 100 Myr

MAZAUD *et al.*<sup>1</sup> have recently analysed geomagnetic reversal time scales, covering the past 100 Myr, and they concluded that the geomagnetic reversal rate has a 15-Myr periodicity superimposed on a monotonic function, which they modelled with a lorentzian function. However, because of the analysis procedure they used, the origin (and hence interpretation) of this periodicity must be questioned. The reversal frequency as a function of time was determined using a fixed-length, rectangular, sliding window: the estimated reversal rate for each position of the window being the number of reversals appearing within the window divided by the window length. Fixed-length sliding window techniques such as this are notorious for producing spurious frequencies when used to analyse time-sequenced data generated by a process which embodies a random element. This occurs because the random component typically has a fairly broadband spectral representation and the fixed-length window acts as a filter which allows through only certain frequencies.

McFadden and Merrill<sup>2</sup> have also analysed geomagnetic reversal time scales and found no indication of a 15-Myr periodicity in the reversal rate. Instead, they concluded that the geomagnetic reversal process is essentially a Poisson process and that since the Cretaceous Normal Polarity Interval the reversal rate has increased approximately linearly with time. Using a maximum likelihood method they concluded that  $\lambda$ , the reversal rate, is given approximately by

$$\lambda = \alpha + \beta t \quad (1)$$

where  $\alpha = 4.41 \pm 0.85$  (Myr<sup>-1</sup>) and  $\beta = -0.051 \pm 0.014$  (Myr<sup>-2</sup>) with  $t = 0$  now and increasing backwards in time. A Poisson process produces randomly distributed interval lengths,  $\tau$ , between events (reversals) with probability density,  $\rho(\tau)$ , given by

$$\rho(\tau) = \lambda \exp(-\lambda\tau) \quad (2)$$

Using a random number generator, random sequences of interval lengths have been generated from the distribution of equation (2) with  $\lambda$  given by equation (1), using the point estimates of  $\alpha(4.41)$  and  $\beta(-0.051)$ . Analysis of such sequences by the method of Mazaud *et al.*<sup>1</sup>, using as they did a window of 4 Myr, results in estimated  $\lambda$  curves strikingly similar to theirs. Autocorrelation of these  $\lambda$  curves (after subtraction of a linear component) gives periodicities very similar to that of Mazaud *et al.*<sup>1</sup>, yet  $\lambda$  contains no periodic component. Using a 2-Myr window, a similar periodicity is still apparent but

buried by the (expected) increased noise. Using an 8-Myr window the amplitude of the periodicity is decreased by the increased smoothing of the longer filter.

Thus, the evidence for the observed 15-Myr periodicity being a real geodynamo phenomenon may not be as convincing as the claim of Mazaud *et al.*<sup>1</sup>, and the possibility that  $\lambda$  has simply increased linearly with time and that the observed periodicity is merely a by-product of the analytical technique must be strongly considered.

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MAZAUD *ET AL.* *REPLY*—McFadden suggests that our report of a 15-Myr periodicity in the frequency of geomagnetic reversals arises from our use of a fixed-length, rectangular window to determine the number of reversals per unit time. We are well aware that the use of such a window can introduce apparent periodicities (even for random data) because the fixed-length window acts as a filter, permitting only certain frequencies to be seen. However, when such a filter does introduce spurious periodicities, the period is directly related to the window width. In our work, we used windows ranging from 1 to 10 Myr. Although the amplitude of our periodicities and the signal-to-noise ratio varied somewhat, the period itself was confined to the narrow range of 13–17 Myr. If the period had merely been the result of using a rectangular window, we would expect a much larger variation in its value.

We now turn to the more important and more interesting point of McFadden's comment, namely, the periodicity in his computer-generated time series. Following McFadden's method we produced over 50 different synthetic time series, which were then analysed by the fixed-length, rectangular-window method. Our goal was to determine whether all of the time series resulted in periodic oscillations of the same period. In our study, the answer was clearly no: while some of the time series did give rise to a distinct periodicity, this was not the general case. In fact, we found an nearly continuous range between time series which had well-defined periodicities and those in which no clear periodicity could be discerned. This result provides additional evidence that the periodicity which we observed in

the reversal frequency did not arise from our method of analysing the data. However, it also shows that a completely random phenomenon can sometimes give rise to a periodic one. With regard to the frequency of geomagnetic reversals, we certainly cannot exclude the possibility that in this particular situation, the periodicity results from a probabilistic process. In this respect, McFadden's criticism has contributed to a deeper insight of our own data.

Finally, note that we never expected that our work should serve as the final word on the nature of the reversal process. The fact that McFadden and Merrill have reached a different conclusion only demonstrates that this is a complex problem whose final resolution requires further work.

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